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

8A. Vitamin A and Cancer

December 1991

By
A. Catherine Ross, Ph.D.

Prepared for
CENTER FOR FOOD SAFETY AND APPLIED NUTRITION
FOOD AND DRUG ADMINISTRATION
DEPARTMENT OF HEALTH AND HUMAN SERVICES
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Life Sciences Research Office
Federation of American Societies
For Experimental Biology
9650 Rockville Pike
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 of 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.

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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

Date

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Director
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I. INTRODUCTION

The possibility of a relationship between vitamin A and cancer first became apparent shortly after vitamin A was recognized as a distinct, new nutritional entity in 1913 (McCollum and Davis). In the 1920s, Mori (1922) and Wollbach and Howe (1925) reported that vitamin A deficiency was associated with a change from normal epithelial morphology to a xerotic, squamous, keratinized epithelium in various tissues including the mucosal linings of the trachea, larynx, and bronchi. Since metaplastic change is often the precursor of malignant transformation, it was reasonable to propose that vitamin A functions in the maintenance of normal tissue morphology and control of cellular growth. Experiments conducted from the mid-1950s to the present have provided strong evidence that natural vitamin A (retinol and its fatty acid esters) and synthetic analogues of retinol, collectively known as retinoids, can regulate cellular differentiation in a variety of experimental systems [see ref. Roberts and Sporn (1984) for review]. Many of the retinoids are potent inhibitors of neoplastic change initiated by chemical carcinogens or other transformants (Roberts and Sporn, 1984), and several of the retinoids have gained importance as therapeutic agents in certain proliferative diseases, mainly of the skin (Bollag, 1983).

A. DIET AND CANCER

The magnitude of the relationship between environmental risk factors and the development of human cancers is still unknown. In 1981, Doll and Peto (1981) estimated that some 80–90 percent of human cancers may be attributable to environmental factors, and that the etiology of about 35 percent (range 10–70 percent) of human cancers may be related to dietary factors. Estimates made by other researchers have been similar (United States Department of Health and Human Services [USDHHS], 1988). Given the hypothesized role of vitamin A in growth control and cellular differentiation, the relationship of vitamin A as a nutrient to cancer incidence and the relationship of vitamin A or its analogues to cancer treatment have become a subject of great interest to biologists, clinicians, and the public.

Several major reports on nutrition and health, summarized below, have reviewed the scientific literature through the late 1980s on which our current understanding of the potential relationships of vitamin A and human cancer is based. These reports form the benchmarks from which this update and review are based. Between 1988 and the present, over 50 new reports have been published on the relationship of vitamin A in the diet, or vitamin A status as determined by serological measures, to indices of cancer risk. The major objective of this chapter will be to assess our current knowledge based on the benchmark references and on these additional human studies and basic experiments.

B. VITAMIN A: NOMENCLATURE AND EXPOSURE

In this review, the term vitamin A will be used in the nutritional sense to describe both dietary retinol and the portion of those dietary carotenoids which, through metabolism, give rise to retinol. Thus, dietary vitamin A comprises retinol and retinyl esters which are obtained exclusively from foods of animal origin or from vitamin supplements and the carotenoids with provitamin A activity that are obtained mainly from foods of plant origin (leafy green and yellow-orange vegetables) or, to a more limited extent, from milk, eggs, or other tissues of animals which have absorbed dietary carotenoids. Of the over 500 carotenoids that have been identified chemically, only about 50 have any vitamin A activity (Bendich and Olson, 1989). In the common leafy green and yellow-orange vegetables, the predominant provitamin A activity is due to a hydrocarbon carotene, beta (β)-carotene, which makes
up 75–85 percent of the total carotenoid in these foods (Bendich and Valez, 1987; Micozzi, 1989; Vitamin Nutrition Information Service [VNIS], 1987). Other members of the carotenoid family such as the oxygenated carotenoids (e.g., lutein, epoxycarotenoids) and other hydrocarbon carotenoids (e.g., lycopene) may be absorbed and are found in the circulation but cannot be converted to retinol and thus do not have provitamin A activity (Micozzi, 1989). It is nearly always desirable to distinguish the individual chemical forms of retinoids or carotenoids; however, this information is not always available or has not been distinguished in published reports. In these cases, the general terms vitamin A, retinoids, provitamin A, or carotenoids will be used.

In the typical U.S. diet, preformed vitamin A constitutes approximately two-thirds to three-quarters of the total vitamin A; the remaining portion is derived from carotenoids with provitamin A activity (National Research Council [NRC], 1989a,b; Ziegler, 1991). In other parts of the world, carotenoids typically provide 80–90 percent of dietary vitamin A (see Underwood in ref. VNIS, 1987).

Few of the studies on diet and cancer published prior to the mid-1980s were intentionally designed to discriminate between the retinol and carotene components of total dietary vitamin A. However, when the results of dietary questionnaires pointed towards the protective effects of foods which are major sources of β-carotene, a potential relationship between dietary or plasma carotene(s) and cancer rates emerged. In 1981, Peto and colleagues (1981) reviewed the literature available at that time and formulated the provocative question "Can dietary beta-carotene materially reduce cancer rates?" Since then, there has been a gradual evolution toward experiments designed to discriminate between the potential capabilities of preformed vitamin A vs provitamin A to modulate human cancer risks. Thus, one goal of this review will be to evaluate the strength of evidence that retinol or β-carotene is independently associated with the risk of human cancer.
II. STATUS OF SCIENTIFIC OPINION: THE BENCHMARK DOCUMENTS

Both the Surgeon General's Report on Nutrition and Health (USDHHS, 1988) and the National Research Council Report on Diet and Health (NRC, 1989a) have considered in detail the information available through 1987 on the relationship of specific nutrients and nutritional status to the development of various types of cancer. Cancers, described as population of cells that have acquired the ability to multiply and spread without the usual biological restraints (NRC, 1989a), may form in nearly all tissues of the body. Collectively, cancers of all sites are the second leading cause of death in the U.S. (USDHHS, 1988); in 1984, cancer accounted for 22 percent of all deaths in the U.S. (USDHHS, 1988). The distribution of cancers within the population and their etiological determinants differ according to individual cancer sites. Most cancers are classified by organ site, while some are classified histologically (NRC, 1989a). Most cancers occur with greater frequency as people age and thus cancer, like several other degenerative diseases, is largely a disease of aging. Cancers which are common in the U.S. and associated with dietary factors include: cancers of the gastrointestinal, digestive tract (esophageal, stomach, pancreatic, and colorectal cancers), cancers associated with the reproductive organs (breast, endometrial, and ovarian cancers in women and prostate cancer in men) and lung, liver, and bladder cancer (NRC, 1989a).

A. VITAMIN A AND CANCER: CONCLUSIONS OF THE BENCHMARK REPORTS

There is substantive agreement, with some difference in emphasis, between the Surgeon General's Report (USDHHS, 1988) and the report of the National Research Council (NRC, 1989c) regarding the evidence relating vitamin A and cancer, as is summarized below. Most of the studies of diet and human cancer have been observational, epidemiological investigations (see further below). Such studies have the ability to weigh associations and correlations but cannot demonstrate a cause-and-effect relationship. Thus, these studies have either provided suggestive evidence of varying strength for a relationship or have not provided evidence of a statistically significant association.

Both the Surgeon General's Report (USDHHS, 1988) and the NRC report (1989a) concluded that the consumption of foods which are high in β-carotene may protect against some epithelial cancers, and both concurred that the evidence is strongest in the case of cancer of the lung. The Surgeon General's Report (USDHHS, 1988) found the evidence relating the consumption of foods high in vitamin A and carotenoids to protection against lung cancer to be strongly suggestive, while the NRC report reached a similar conclusion regarding foods rich in β-carotene that was stated somewhat more guardedly. The evidence upon which these reports were based regarding preformed vitamin A was, indeed, inconsistent. The weight of the evidence did not support a significant, positive association between the amount of preformed vitamin A in the diet or the levels of retinol in blood and reduced cancer rates.

B. SIGNIFICANT RECENT DEVELOPMENTS

Since the early 1980s, the concept of retinol and β-carotene as distinct entities and distinct exposures in experimental studies has gained recognition and importance (NRC, 1989a; Ziegler, 1991). In these benchmark reports, retinol and β-carotene were discussed as distinct exposures which can be evaluated independently as risk factors in epidemiological and experimental studies. Since the conduct of the studies that were reviewed in 1987–88, there has been considerable progress in understanding the fundamental metabolism and functions of retinol and its metabolites. There has also been substantial improvement in the food composition database regarding carotenoids (Lachance, 1988;
Micozzi, 1989; Ziegler, 1991) which has been critical for analysis of the separate effects of dietary provitamin A or non-provitamin A carotenoids. These recent developments will be commented upon briefly before reviewing the new human studies on vitamin A and site-specific cancers.

1. **Vitamin A: metabolism**

The general features of the metabolism of retinol and carotene have been described previously (Goodman, 1984a; NRC, 1989d; Peto et al., 1981; USDHHS, 1988; VNIIS, 1987) and will be discussed only briefly here. Dietary retinol is absorbed relatively efficiently into the intestinal mucosa where it is esterified with long-chain fatty acids and incorporated into the chylomicron for transport from the intestine to the circulation. Two mechanisms for retinol esterification in the intestine have been proposed (Blomhoff et al, 1990; Ong et al., 1987), and recent evidence from biochemical studies has pointed to an important role of one of the intracellular retinol-binding proteins in the esterification of intestinal retinol (Ong et al., 1987). After secretion from the intestine and movement to the circulation, the majority of chylomicrons containing newly absorbed retinyl esters are rapidly taken up by hepatocytes, most likely via a newly described receptor (Herz et al., 1990). It has also been demonstrated recently that a smaller portion of chylomicron vitamin A is taken up by other organs (Hussain et al., 1989); this means of distribution of vitamin A may have relevance for differentiation in non-hepatic tissues. In liver, newly absorbed vitamin A undergoes a series of metabolic steps leading to deposition of vitamin A (as retinyl esters) in hepatic stellate cells (Blomhoff et al., 1990). When retinol is mobilized from the liver to the circulation, it is secreted in association with a specific transport protein, retinol-binding protein (RBP) (Goodman, 1984b).

Numerous reviews have emphasized that the concentration of retinol and RBP in plasma is under tight homeostatic regulation and does not vary measurably with an individual's vitamin A status, except in extremes of vitamin A deficiency. The concentration of retinol and RBP does vary with age and gender (Pilch, 1985), in diseases of the liver and kidneys (Goodman, 1984b) and with the use of certain hormones such as are found in oral contraceptives (Goodman, 1984b; Pilch, 1985). When retinol intake was varied through the use of vitamin A supplements, small but significant increases in serum retinol and RBP were observed (Wald et al., 1985). The administration of certain synthetic retinoids has been observed to rapidly lower the plasma retinol concentration of animals whose vitamin A nutritional status was normal (Formelli et al., 1987), suggesting interference with a normal, regulatory step in vitamin A transport.

The efficiency with which dietary β-carotene is utilized is more variable. Carotenoids in foods require sufficient bile salts and fat for effective digestion and emulsification prior to uptake into the intestine (Underwood, 1984). Despite the seeming ability of one β-carotene molecule to generate two molecules of retinol, the nutritional value is actually much lower (6 μg of β-carotene is nutritionally equivalent to 1 μg of retinol). The efficiency of carotene absorption decreases somewhat as intake increases (Brubacher and Weiser, 1985). Likewise, there is evidence that the cleavage of β-carotene to retinol in the intestinal mucosa becomes less efficient as intake increases (Underwood, 1984). A portion of β-carotene is cleaved and converted in the mucosa to retinol which then follows the same pathways of transport and metabolism as preformed dietary retinol. Human beings also absorb an appreciable amount of unmodified carotenoids, including β-carotene and other carotenoids which lack vitamin A activity. Like retinyl esters, these carotenoids are also transported from the intestine to blood in the chylomicron and are thought to be rapidly cleared into tissues.

In contrast to retinol, the plasma concentration of carotenoids is sensitive to dietary intake. In individuals on a typical diet, only about 15–30 percent of plasma total carotenoid is β-carotene; this plasma pool represents some 1 percent of total body carotenoid (Bendich and Olson, 1989). Most carotenoids circulate in association with low-density lipoproteins (LDL), which may help to explain
the observed association between concentrations of plasma carotenoids and lipids (e.g., cholesterol). It is also likely that tissue uptake of carotenoids is determined by the interaction of LDL with tissue lipoprotein receptors. A substantial portion of total body carotene is stored in adipose tissue, with lesser amounts in liver and other tissues (Bendich and Olson, 1989). Recent analysis of human serum and adipose tissue has shown that lutein, cryptoxanthin, lycopene, α- and β-carotene are present in both (Parker, 1989).

When supplemental β-carotene was administered in amounts (50 mg/d) far exceeding usual dietary intake (=ca. 1.5 mg/d (Lachance, 1988)), the plasma concentration of β-carotene increased 10-fold (Nierenberg et al., 1991). There was, however, no change in plasma retinol (Nierenberg et al., 1991).

The factors other than diet that determine plasma β-carotene levels are becoming better understood. In a correlation study, Nierenberg et al. (1989) found that plasma β-carotene concentrations were greater in women than men and were inversely related to cigarette smoking and an index of body fatness. The effect of smoking of plasma β-carotene concentration was seen across four intervals of dietary β-carotene (Nierenberg et al., 1989).

2. Vitamin A: functions

Studies over a number of years have implicated a metabolite of retinol, retinoic acid, in the regulation of cellular differentiation. A most exciting and important breakthrough in understanding the mechanism of action of vitamin A began in 1987 with the discovery of nuclear receptor proteins that bind retinoic acid (Giguere et al., 1987; Petkovich et al., 1987). These retinoic acid receptors (RAR), which are highly similar in structure to the receptors that mediate steroid hormone effects, have been described in a number of tissues and are thought to have important regulatory roles during embryogenesis and in maintaining the differentiated state of more mature cells [see ref. (Ross, 1991; Wolf, 1990) for reviews]. Only a very few of the genes that are regulated by the RARs have been identified. As further data become available, a much improved understanding of the relationship between vitamin A (as retinoic acid) and cancer is likely to follow rapidly. There is a low but measurable concentration of retinoic acid in plasma (DeLeenheer et al., 1982); however, it is generally thought that it is the intracellular generation of retinoic acid [through oxidation of retinol (Napoli and Race, 1987)] that is related to maintenance of the normal phenotype and function of cells.

A very recent finding strongly implicates one form of RAR (RAR-α) and, by extension, retinoic acid with one type of cancer, acute promyelogenous leukemia. It was noticed that the position of a breakpoint on human chromosome 17 that is specifically associated with acute promyelogenous leukemia maps is in the same region of the chromosome as the RAR-α. Recent reports (Chang et al., 1991; Chen et al., 1991) by several groups have provided evidence that the RAR-α gene is frequently disrupted by this chromosomal break and translocation to chromosome 15 in patients with acute promyelogenous leukemia. Aberrant transcription of the RAR-α gene leading to a dysfunction of retinoic acid in cell regulation is thus implicated in the development of this form of leukemia. It is very interesting that treatments with vitamin A or other retinoids in pharmacologic quantities have had some success in producing remissions of leukemia (see Castaigne et al., 1990).

It is possible that carotenoids play their putative role as anti-cancer agents after conversion into retinol or in a fundamentally different way from retinol. Beta-carotene has been shown in chemical studies to have antioxidant properties (Burton, 1989) which are not shared by retinol (Bendich and Valez, 1987). The eleven conjugated double bonds of β-carotene enable it to efficiently quench singlet oxygen or free radicals generated through chemical or photochemical reactions, thereby breaking chains of oxidative reactions (Bendich and Valez, 1987; Burton, 1989). Although this property has led to the frequent categorization of β-carotene as an antioxidant vitamin, it is important to keep in mind
that the evidence for this role under physiological circumstances is quite limited (Bendich and Olson, 1989) and that there are other potential means by which this molecule could exert biological activity. For instance, \( \beta \)-carotene may modulate membrane properties important to cell–cell signaling or possibly serve as an intracellular precursor to retinoic acid (Napoli and Race, 1988). It seems likely that the physiologically important concentrations of both retinol and \( \beta \)-carotene are those within cells and that plasma concentrations, although accessible, may have a limited relationship to intracellular events.

3. **Vitamin A: pharmacodynamics**

Differences in the pharmacodynamics of carotenoids vs retinoids were highlighted in an earlier review by Peto et al. (1981), and some of these differences have already been mentioned above. Evidence from animal studies has indicated that the rate of utilization of retinol varies with vitamin A intake. Following the initial report by Lewis et al. (1981), which indicated that retinol recycles between liver and other tissues before it is irreversibly degraded, recent work has confirmed and quantified the extent of retinol recirculation (Green et al., 1987; Lewis et al., 1990). In contrast, there is no evidence for a similar reutilization of RBP. The availability of exogenous retinoic acid can reduce the irreversible degradation of retinol (Keilson, 1979). While there is as yet no direct evidence for such recycling of retinol in man, it is most likely that a similar basic process exists. Thus, while plasma retinol concentrations are nearly invariant over a range of liver retinol reserves, the flux through the plasma compartment and between tissues is related to some aspects of vitamin A status.

The dynamics of \( \beta \)-carotene transport and utilization are less well known. Major unanswered questions concern the local metabolism of carotenoids within human tissues and the resolution of whether \( \beta \)-carotene or other carotenoids have physiological roles outside of those associated with vitamin A. Regarding local metabolism, Napoli and Race (1988) have demonstrated that rodent tissues in vitro are capable of converting \( \beta \)-carotene to retinoic acid, presumably with retinaldehyde as an intermediate, but by-passing formation of retinol. If this pathway also functions in vivo then there may be two, distinct, intracellular precursors of retinoic acid, retinol, and \( \beta \)-carotene, and the production of retinoic acid from these precursors may be regulated separately.

4. **Dietary assessment of vitamin A**

The interpretation of many of the studies which have reported a linkage between consumption of carotene–rich foods and cancer rates have been limited by weakness in the food composition databases with regard to the carotenoid composition and content of specific foods. Additionally, some food–frequency questionnaires have not adequately probed the consumption of foods rich in provitamin A carotenoids vs carotenoids without provitamin A activity. Recent improvements in analytical methods have been important in strengthening the food composition database. As noted by Ziegler (1991), the older, approved spectrophotometric method did not resolve \( \beta \)-carotene from several chemically similar carotenoids. Newer HPLC methods have provided the necessary resolution. Using HPLC, all the green, leafy vegetables were found to contain the same profile of carotenoids but at varying concentrations (Micozzi, 1989). The \( \alpha \)- and \( \beta \)-carotene contents of these vegetables equaled only 9–18 percent of total carotenoids while the majority were oxygenated carotenoids (Micozzi, 1989). The yellow–orange vegetables, in contrast, contained predominantly \( \alpha \)- and \( \beta \)-carotene (Micozzi, 1989).

Considerable uncertainty exists regarding the bioavailable content of carotenoids because of their inherent instability to light, oxygen, and heat during storage, cooking, or other culinary processes (Lachance, 1988). Nonetheless, improvements in the database constitute an important step towards developing valid tests of whether fruits and vegetables, or \( \beta \)-carotene per se, confer protection against
cancer. Lachance (1988) has used information on both the β-carotene content of various vegetables and fruits in the U.S. diet and their per capita disappearance to estimate the major dietary contributors of β-carotene. According to these estimates, just 5 food items contribute nearly 85 percent of the β-carotene in the average diet. These include carrots, sweet potatoes/yams, tomatoes, melons, and spinach. Notably also, a number of green vegetables and most fruits have a low content of β-carotene (Lachance, 1988).
III. VITAMIN A AND HUMAN CANCER

A. TYPES OF STUDIES: METHODOLOGICAL CONSIDERATIONS

Most of the studies conducted before and since 1987 have been epidemiological, observational studies of the case-control design. A number of recent reviews have addressed the types of designs used in epidemiological research and the strengths and limitations of each (Boone et al., 1990; DeWys et al., 1986; Mettlin, 1988; Vogel and McPherson, 1989). Correlational studies can be used to identify ecological associations between a potential risk factor and disease. Case-control studies involve retrospective identification of persons with particular disease characteristics and comparison of that individual's diet or other habits with those of one or more individuals without the disease who have been matched for potentially confounding characteristics (usually gender, age, and other characteristics thought to influence the variables under study). Controls may be hospital controls, that is patients in the same setting but with disease unrelated to the one under investigation, or they may be community (population) controls chosen from the same general population as the cases. Results are usually expressed as the relative risk (ratio of cases to controls) or an odds ratio (ratio of risks). Trends are examined by testing the significance of the dose-response gradient across categories of an exposure (e.g., tertiles of vitamin A intake) for relative risk.

As noted by Mettlin (1988), dietary exposures in such retrospective studies are usually measured by questionnaire and interview, requiring recall of dietary habits across extended intervals, thus exposures are observed post hoc. In the experiments to be reviewed, the primary data in nearly all studies have been information on food items or groups of foods from which secondary data on nutrient composition have sometimes been derived.

Prospective studies provide a higher level of epidemiological evidence and seek to correlate nutrient intake and disease occurrence. The success of such studies depends on their ability to study a range of intakes (Vogel and McPherson, 1989); therefore, a study population with dietary heterogeneity best serves this type of design.

Intervention trials or clinical trials are truly experimental, providing an opportunity to compare treatment and control groups. Such studies are usually preceded by significant results in case-control or prospective studies and by pilot or feasibility work to determine the size of the study necessary for statistically meaningful results and, perhaps, operational logistics. Although intervention trials could involve true dietary modification, those that have been conducted recently have examined the effects of supplemental nutrients on disease outcome. The process by which potential chemopreventative agents, including β-carotene and certain retinoids, have been identified and moved towards clinical trials has been reviewed recently by DeWys et al. (1986) and by Boone et al. (1990).

B. VITAMIN A AND CANCER: BY SITE

As noted in both the summaries of the Surgeon General's Report (USDHHS, 1988) and the National Research Council's report, Diet and Health (NRC, 1989a), the strongest evidence for a protective effect of vitamin A, principally in the form of β-carotene, has been found previously for cancer of the lung. Seven studies since 1987 that address dietary or plasma vitamin A and lung cancer will be reviewed first. Carotenoids and/or vitamin A have also been postulated to protect against epithelial cancers at sites other than the lung. New studies have been reported which have addressed the protective effect of diets including vitamin A, carotenoids, and β-carotene on cancers of the mouth and pharynx, esophagus, stomach, pancreas, and colon or rectum. These will be considered together as cancers of
the gastrointestinal-digestive tract. Other studies have addressed cancers with a hormonal relationship, namely, cancers of the breast, ovary, endometrium and cervix in women, and cancer of the prostate in men. Finally, studies of cancers of the bladder and skin will be considered.

All of the human epidemiological studies considered in this text have been summarized by organ site in Appendix Table I.

1. **Lung cancer**

Lung cancer is the leading cause of cancer mortality among men in the U.S. and most technologically advanced countries and is approaching equality with the rate of breast cancer for women (NRC, 1989a). The most important causal factor for both men and women is cigarette smoking; certain occupational exposures also contribute to lung cancers in men (NRC, 1989a). The strongest evidence for a role of vitamin A in the prevention of human cancers has come from epidemiological investigations which have correlated the consumption of foods rich in preformed vitamin A or provitamin A and the risk of lung cancer (USDHHS, 1988). The Surgeon General's Report of 1988 summarized results from 8 case-control studies and 4 prospective studies published between 1978 and 1987 that examined the relationship of foods (leafy green vegetables) or nutrients (total vitamin A, retinol, total carotenoids, or β-carotene) and lung cancer rates (measured as incidence or death). A consistent observation was a greater relative risk of lung cancer, after adjustment for smoking habits in nearly all studies, in individuals whose nutrient level was in the lowest category (lowest half, tertile or quartile) in comparison to those whose nutrient level was in the highest category. Generally, the relative risk (lowest category of intake compared to the highest category of intake) was about 1.7–2.0. Recent reviews by Colditz et al. (1987), Willett (1990), and Ziegler (1991) have also presented summaries and critiques of these data and some of the more recent studies on dietary vitamin A and lung cancer.

Despite the overall consistency of these earlier studies, there were discrepancies which limited the inferences that could be drawn. All were observational studies which, inherently, could not distinguish between associations and causal relationships. The studies varied considerably in the form and quality of interview and dietary data, whether use of vitamin A supplements was considered, in the number of cases examined, in the detail to which smoking habits were examined, and in whether or not disease was classified by histological type. In some studies the dietary data were consistent with a protective effect of foods rich in β-carotene, while in others the protective relationship was also seen with dietary or supplemental retinol or with the use of certain vegetables (e.g., tomatoes, cruciferous vegetables) that are not good sources of β-carotene. Studies have differed with respect to whether men and women appeared to be equally protected, whether protection was limited to, or more likely for, squamous cell carcinoma vs adenocarcinoma, and in whether or not dietary retinol was also protective. Thus, collectively these studies supported the conclusion that a diet high in leafy green and yellow-orange vegetables and fruits is generally protective against cancers of the lung. It was unlikely, but not disproved, that this protection was due to dietary retinol. The distinct contributions of dietary β-carotene, other carotenoids, or other factors concentrated in carotenoid-rich foods could not be clearly discerned.

Connett et al. (1989) reported results from a case-control study of the relationship between baseline nutrient intake, serum nutrient levels, and cancer mortality among participants in the Multiple Risk Factor Intervention Trial (MRFIT). Of the 156 initially healthy men who subsequently died from cancers, 66 died of lung cancer; all of these were current or past smokers. Controls matched for age, smoking status, and participation variables were chosen from the study survivors. For all cancers, there were no significant differences in serum levels of total carotenoids, β-carotene, retinol, RBP, or α-tocopherol. However, for lung cancer cases, serum total carotenoids were significantly lower in cases
than controls. A similar tendency was observed for serum \( \beta \)-carotene levels, but these did not differ significantly from controls. No differences were found for retinol or RBP. Dietary intake of vitamin A, \( \beta \)-carotene, retinol, vitamin E, and cholesterol were calculated from a 24-hour dietary recall taken at baseline. Intake of \( \beta \)-carotene was about 25 percent lower in lung cancer cases, but this difference was not significant. The authors recognized that a single 24-hour dietary recall is generally a poor measure of usual dietary intake. Thus, to the extent that serum carotenoid levels and a 1-day dietary record reflected dietary patterns, this study was consistent with an inverse relationship between carotenoid intake and risk of lung cancer. However, the low serum total carotenoids may also have been related to subtle differences in smoking habits or to dietary differences related to smoking habits.

Bond et al. (1987) conducted a case-control study of 308 former employees of a Texas chemical company who had died of lung cancer between 1940 and 1980 in comparison to matched living or decedent controls. The frequency of consumption of 29 food items was determined by interviews with subjects (for controls) or next-of-kin (for all cases and some of the controls). Standard food portion sizes were assumed, and a vitamin A index and a carotenoid index were calculated. After adjustment for smoking, vitamin supplement use, and education, vitamin A intake was inversely associated with lung cancer risk. The effect was strongest in comparison to the living controls and appeared to be greatest for heavy smokers. Similar odds ratios were found when the dose-response relationship of carotenoid intake and lung cancer was examined. When the frequency of consumption of specific food items was analyzed, there was no association with consumption of squash or sweet potatoes (rich in \( \beta \)-carotene), but there was an inverse association with consumption of carrots and melon (also rich in \( \beta \)-carotene) and with tomatoes (low in \( \beta \)-carotene) and with consumption of certain low-fat foods. The results of this study were generally in agreement with the previous conclusions that frequent consumption of fruits and vegetables is protective against lung cancer. Due to the long interval between case death and the collection of much of the dietary data by proxy there were obvious concerns about the reliability of the dietary information and the potential for misclassification. In a validation study conducted 3-5 months later in a subset of respondents, there was less than 50 percent agreement with the original response category for the frequency of use of 4 food items which would contribute significantly to the vitamin A or carotenoid indices.

A large case-control study of lung cancer incidence in a high-risk region of southern Louisiana was reported by Fontham et al. (1988). Twelve hundred fifty-three lung cancer cases were compared to 1274 hospital controls. The monthly frequency of consumption of 59 food items prior to onset of illness was determined by questionnaire, and cancer rates were analyzed by race, gender and histological type. The consumption of fruits and vegetables was lower in cases than in controls. For carotene intake, there was an inverse relationship between relative risk and tertiles of intake in cases of squamous cell and small-cell carcinomas, but the trend was not significant. In contrast, the inverse gradient for vitamin C was highly significant. Because intakes of carotene and vitamin C were strongly correlated (\( r = 0.64 \)), it was not surprising that adjustment for vitamin C eliminated the effect of carotene. However, the opposite adjustment left the effect of vitamin C intact. Although most studies have not supported a relationship of vitamin C and lung cancer (NRC, 1989a), the authors pointed out that vitamin C intake in this population is generally low so that effects of higher intakes may have been more readily discerned. Overall, the results were most consistent with a strong protective effect for fruits, a weaker effect for vegetables, and only a modest indication that carotenoids were protective. Retinol showed little association except in cases of adenocarcinoma for which there was a significant inverse relationship, especially among black men and women whose diets contained more preformed retinol from organ meats (livers).

Koo (1988) reported results of a case-control study of lung cancer among women in Hong Kong who had never actively smoked. Eighty-eight cases of lung cancer, by histological type, were compared with 137 population controls (Koo, 1988; Koo et al., 1987). Because of the difficulty in quantifying the intake of Chinese foods from mixed dishes, women were queried only about their frequency of use of
certain foods. In this study, more frequent consumption of fresh fruit or fresh fish conferred protection against lung cancer. There were inverse trends between tertiles of intake of both β-carotene and retinol for all cases combined but these were not statistically significant. However, trends approached statistical significance for both β-carotene and retinol intakes in women with adenocarcinoma or large-cell lung cancer. The strongest inverse trend was found for a composite indicator of a "good diet" (based on frequency of consumption of cruciferous vegetables, fresh leafy green vegetables, carrots, beans/legumes, tofu/soy, fresh fruit, soup, milk, and fresh fish). Thus, the weight of evidence from this unique study supported a protective role of fresh fruits and a diet pattern containing a variety of fresh fruits and vegetables. These effects were observed among individuals in whom tobacco smoking was not a risk factor.

Le Marchand et al. (1989) reported results of a case–control study of lung cancer among men and women in Hawaii. Lung cancer cases (230 men and 102 women) were matched by age and gender to population controls (597 men and 268 women). A diet history was obtained by home interview using a quantitative food–frequency questionnaire designed to probe consumption of foods which provided greater than 85 percent of the intakes of carotene, retinol, vitamin C, and other nutrients. The use of nutrient supplements, tobacco, and alcohol was also queried. Most subjects were interviewed directly but proxy interviews (with next–of–kin, usually the spouse, who had lived with the case subject for at least 5 years) were obtained for 29 percent of the cases and 7 percent of the controls. In this study, total vitamin A (foods plus supplements) was inversely related to lung cancer risk for both sexes. Of total vitamin A, only β-carotene intake was inversely related; there was no relationship for retinol or supplements. The protective effect of total vitamin A, β-carotene, and other carotenoids with vitamin A activity showed consistency across tertiles of intake, with gender, among ethnic groups, and with histological classification. Protection was observed for both men and women who had reported ever smoking. All vegetables, including several not rich in β-carotene, showed a stronger inverse relationship with risk than was found for β-carotene. The results of this study were consistent with a protective effect of carotenoids but pointed more strongly to a beneficial effect of a diet rich in vegetables. As noted by the authors, the constituent(s) most strongly associated with lowering risk may be common to all the food types; components of vegetables other than β-carotene were also implicated by the results of this study.

Jain et al. (1990) reported an analysis of dietary data from men and women in the Toronto area who had a histologically–confirmed diagnosis of lung cancer. The estimated intake of 34 nutrients was assessed from a diet history conducted by a trained interviewer in which the frequency and the usual intake of 81 food items chosen to cover the complete intake of both retinol and β–carotene, and of cholesterol. A detailed smoking history and occupational history were also obtained, and the very strong association of smoking with lung cancer was confirmed by this study. Regarding diet, there was a significant inverse association with vegetable intake but there were no significant relationships with consumption of fruit, total vitamin A from foods, or the intakes of retinol or β–carotene. The study did reveal a significant, inverse, association with cholesterol intake and a strong inverse association with the consumption of nitrate, primarily from certain vegetables (lettuce, broccoli, spinach, and beets), some of which are also rich sources of β–carotene. Thus, this study did not lend additional support to the hypothesis that dietary vitamin A or β–carotene is protective against cancer of the lung and, instead, provided further evidence that constituents of vegetables, other than β–carotene, may be significant in reducing the risk of lung cancer.

A case–control study reported by Dartigues et al. (1990) examined the relationship of dietary intake of preformed vitamin A and β–carotene to risk of lung cancers classified histologically as epidermoid. One hundred forty-three cases of lung cancer admitted to 6 urban hospitals in southwestern France and 290 hospital controls were selected; of these, 106 cases and 212 controls were matched, interviewed, and complete data were obtained for analyses. Cases and controls were mostly men over the age of 60 who were farmers or blue–collar workers for whom tobacco and alcohol use was stated
to be high. The exposure factor used in the analysis was the average vitamin A and β-carotene intake during the 6 months preceding the interview and the average intake over the past 10 years when the interview indicated that diet had changed during the preceding 6 months. The authors considered recall bias to be the most likely source of information bias in this study. The median daily intakes of preformed vitamin A were 600 µg (retinol equivalents, RE) in cases and 1020 in controls while the medians for β-carotene were 1510 and 2170 RE, respectively, yielding odds ratios of 4.3 for preformed vitamin A and 4.1 for β-carotene. However, the trend with increasing consumption of either form of vitamin A was not significant. The results of this study are consistent with a protective effect of β-carotene on the risk of developing epithelial lung cancer and also suggest that a higher intake of preformed vitamin A may also be protective. The authors commented that consumption of preformed vitamin A from dairy products is relatively high in this population as compared to some of the populations that have taken part in U.S. studies of diet and lung cancer.

In a prospective study, Paganini-Hill et al. (1987) followed a cohort of over 10,000 residents of a California retirement community for 5 years during which 643 new cancers were diagnosed. Of these, 56 were cancers of the lung. At baseline, information was obtained on the frequency of consumption of 59 food items that were subsequently used to construct a quantitative index of vitamin A and β-carotene intake. Information was also obtained on vitamin supplement use, smoking habits, and personal habits. The results for 56 lung cancers were analyzed according to tertiles of intake for dietary vitamin A, dietary β-carotene, supplemental vitamin A, and total vitamin A. No significant differences or trends in the age-adjusted incidence rates were observed by level of nutrient intake for all cancers combined or for lung cancer specifically. The authors noted that this population is generally well-fed so that even those in the lowest tertile did not have an absolutely low nutrient intake.

This study (Paganini-Hill et al., 1987) also illustrated some of the significant factors that may confound interpretation of the vitamin A-cancer relationship. The investigators examined whether vitamin A intake differed substantially with smoking habits in this population. Indeed, current smokers were more likely to be in the lowest tertile for vitamin A intake than either past smokers or those who had never smoked. The investigators also examined whether the report of dietary habits agreed over time. Some participants were asked to complete a second questionnaire; the agreement between reports at baseline and after 5 years was only 50 percent for the intake of β-carotene and 72 percent for the intake of vitamin A from supplements.

Thus, this prospective study, in which information on diet and supplement use was collected in a comparatively thorough and quantitative fashion, did not lend support to the hypothesis that either dietary vitamin A, retinol, or β-carotene is protective against cancer of the lung.

Stähelin et al. (1987) reported results of a prospective study in which plasma nutrients were measured in 1971–73 in a cohort of male employees of Swiss pharmaceutical firms and follow-up was continued until 1980. Of 268 deaths, 37 were attributed to lung cancer. Plasma β-carotene levels, without adjustment for smoking, were significantly lower for all cases including lung cancers separately. No dietary data were provided. The emphasis of this report was not on vitamin A or carotenoids, and analysis of the β-carotene data by smoking status was not presented in sufficient detail for evaluation. Since plasma nutrient concentrations were measured near the time of blood collection, nutrient losses during sample storage were not a major concern for this study.

Kune et al. (1989) reported that the serum concentrations of retinol and β-carotene were significantly lower in 72 men consecutively diagnosed for lung cancer at a Melbourne, Australia, hospital than for 73 consecutively admitted hospital controls. Neither the extent of cancer (local, regional, or metastatic) nor the type of cancer (squamous, adenocarcinoma, large cell or small cell) significantly influenced these results.
In a study of similar design conducted in Louisiana, LeGardeur et al. (1990) examined the serum levels of vitamin A, β-carotene, RBP, vitamin E and vitamin C in 59 cases (57 men and 2 women of which 51 percent were black and 49 percent white) with newly diagnosed, histologically or cytologically confirmed, primary lung cancer. Cases were compared to both hospital and community controls. In comparison to the hospital controls (only 11 percent of whom had never smoked) the lung cancer cases had significantly lower levels of carotenoids, vitamin E, vitamin C, and cholesterol, but differences in serum vitamin A were not significant (p = 0.07). On average, the hospital controls also had lower levels of each serum nutrient, except cholesterol, than the community controls, although the only significant difference was for vitamin C. With the designs of these studies, it could not be determined whether the lower concentrations of serum retinol (Kune et al., 1989) and carotene (Kune et al., 1989; Le Gardeur et al., 1990) reflect manifestations of cancer or may be risk factors predisposing to disease.

Knekt et al. (1990) reported the results of a prospective serological study which examined the association between serum retinol, RBP and β-carotene levels and the subsequent incidence of cancer in a large cohort (consisting of 25 smaller cohorts which made up the Finnish Mobile Clinic Health Examination Survey carried out in 1968–72). Information on smoking habits, occupation and parity was obtained, height and weight were determined and a blood sample was drawn for storage of serum (−20°C). Of 36,265 men and women, ages 15–99, who had no prior history of cancer at baseline, 766 cancer cases (all sites) developed over an 8-year follow-up period.

At baseline, the mean age for cases and controls was identical (57 yr). Each case was then matched with two controls by age, sex, and municipality. Of cases, 39.7 percent were smokers at baseline as compared to 28.9 percent of controls. For 453 male cases and matched controls, mean serum retinol concentrations equalled 645 µg/L for cases and 667 µg/L for controls; this difference was statistically significant, as were differences for RBP (58.6 and 60.2 mg/L) and for β-carotene (72.3 and 84.1 µg/L). For females, the comparable values showed the same pattern but did not differ as greatly and were not statistically significant. When data were adjusted for smoking, there was a significant, inverse relationship between relative cancer risk for men and the concentrations of serum retinol, RBP and β-carotene. Differences for women were not significant. When case–control differences were computed for men according to cancer site differences were highly significant for lung cancer (p<0.005) and for "cancers related to smoking" (defined as cancers of the lip, oral cavity, pharynx, esophagus, respiratory organs and urinary bladder)(p<0.001). No other site–specific differences were significant. Similarly, for RBP there were significant case–control differences for cancer of the lung, urinary organs and cancers related to smoking, while for β-carotene differences were significant for cancer of the lung and for cancers related to smoking. Low β-carotene at baseline was also significantly related to increased risk of prostate cancer in men and of breast cancer in women. The associations observed in this study were generally greater during the first 2 years of follow-up, suggesting that the lower values in cases may reflect the existence of occult, preclinical disease at baseline.

Concern has been raised previously about the validity of vitamin A, and particularly β-carotene, assays on serum which has been stored for years; however, it appears that, at least, all sera were treated comparably in this study. Thus, this study, which is notable for its large case number, provides evidence that serum β-carotene, and to a lesser extent retinol, is inversely associated with cancer risk, particularly with smoking–related cancers.

In summary, the results of six (Bond et al., 1987; Connett et al., 1989; Dartigues et al., 1990; Fontham et al., 1988; Koo, 1988; Le Marchand et al., 1989) of the eight recent studies on lung cancer that included dietary information are generally consistent with the earlier literature. Most of the studies have provided evidence, in the form of a statistically significant association or a non–significant tendency, that consumption of carotene–rich foods is associated with some protection against cancer of the lung. Three of the studies (Bond et al., 1987; Koo, 1988; Le Marchand et al., 1989) also found
evidence consistent with a protective effect of retinol, while three studies did not (Connett et al., 1989; Fontham et al., 1988; Paganini-Hill et al., 1987). Thus, while the majority of previous and recent studies have not pointed to a protective effect of a high intake of retinol, the strength of this inference has been weakened somewhat by two of the recent studies. The most consistent finding among all studies was the protective quality of diets in which fruits and vegetables are regularly consumed. Upon further analysis by nutrient content, the evidence supports a role(s) for a variety of nutrients including carotene, vitamin A in some studies, vitamin C in some studies, and, most likely, other constituents that are also concentrated in leafy green, cruciferous and yellow-orange vegetables and fresh fruits. Thus, the question of a specific protective effect for β-carotene in the usual diet in prevention of lung cancer is still open.

The interpretation of the serological studies is complicated by the use of stored sera in some investigations and uncertainty as to whether differences in serum nutrient levels are related to diet, other health-related habits (e.g., smoking), metabolic differences that precede cancer, or other factors. The relationship of serum β-carotene to smoking status is a strong one and may mediate the differences between cases and controls observed in these studies.

The Surgeon General's Report (USDHHS, 1988) has listed eight ongoing, clinical prevention trials in a variety of populations which have been designed to critically test the ability of supplemental β-carotene to protect against cancers of the lung. The results of these trials should become available within the next few years.

2. Head and neck (oral/pharyngeal, laryngeal, and esophageal) cancers

Oral/pharyngeal cancers (cancers of the head and neck) are strongly associated with the use of tobacco and alcohol (McLaughlin et al., 1988). Recent case-control studies have addressed whether dietary factors including vitamin A and carotenoids influence risk of oral/pharyngeal cancer, and a short-term intervention trial has tested the ability of supplemental β-carotene or β-carotene plus retinol to prevent progression of oral leukoplakia. A clinical trial of 13-cis-retinoic acid in chemoprevention has been reported recently.

McLaughlin et al. (1988) conducted a large case-control study in 4 areas of the U.S. in which 871 cases of oral cancer were matched with 979 population controls. This report examined only the white members of a study population (83 percent of total cases) that also included non-white and Hispanic cases. The frequency of consumption of 61 food items was determined and standard portion sizes were assumed to construct indices of nutrient intake. Supplement use was not included. The results of this study showed an inverse relationship between the intake of fruit and the risk of oral and pharyngeal cancers. Results were consistent for men and women. Individuals in the highest quartile for intake had about half the relative risk of those in the lowest quartile. However, the authors noted that the vitamin C, carotene, or fiber contents of fruits did not appear to fully account for this relationship because these nutrients in vegetables did not provide similar protection.

Franco et al. (1989) reported results from a case-control study conducted in Brazil of 232 men and women with newly diagnosed cancers of the oral cavity compared to hospital controls. An interview-questionnaire was used to ascertain frequency of consumption of broad groups of foods including carotene-rich foods (carrots, pumpkins, and papaya) as well as citrus fruits and green vegetables. Significant reductions in risk were associated with more frequent consumption of carotene-rich foods and citrus fruits. After adjustment for smoking and alcohol use, the association with carotene-rich foods was marginally significant, and that with citrus fruits was statistically significant. There was no association with the intake of green vegetables. Thus, both the studies of McLaughlin et al. (1988) and Franco et al. (1989) are consistent with a protective effect of fruits which
may be related, in part, to their content of carotenoids. However, it seems most likely that other constituents or a combination of constituents contributed to this protective effect.

In 1988, Stich et al. (1988) reported results of a 6-month intervention trial in which supplemental \( \beta \)-carotene or vitamin A was provided to Indian men with well-developed oral leukoplakia. These men were from a population that habitually chews betel nut (areca) and has a high rate of alcohol use. Thirty-five men were supplemented with \( \beta \)-carotene (180 mg/wk), 60 with an equal dose of \( \beta \)-carotene plus vitamin A (100,000 IU/wk), and 35 with a placebo capsule. The habitual use of betel nut continued during the study. No adverse reactions due to treatment were reported. However, the development of skin yellowing in the \( \beta \)-carotene-supplemented men prevented the trial from remaining double-blind. The appearance and size of leukoplakia and the percent of micronucleated cells were evaluated after 3 and 6 months.

By 6 months, there was significant reduction in appearance and size of oral leukoplakia in the group supplemented with both \( \beta \)-carotene and vitamin A and a marginal reduction in those supplemented with \( \beta \)-carotene alone. The percent of micronucleated cells, an indicator of the genotoxic activity of areca constituents, was reduced very significantly after 3 months with either supplement. This trial supported the therapeutic efficacy of supplemental \( \beta \)-carotene and vitamin A in the regression of oral leukoplakia, even during continued exposure to genotoxic agents.

Recently, de Vries and Snow (1990) have compared the serum vitamin A, vitamin E, and \( \beta \)-carotene levels in 71 patients with a single primary head and neck tumor to 15 patients with a recurrence (second primary tumor). No other information regarding the two groups of patients was provided. The results of serum analysis revealed that both serum vitamin A and vitamin E, but not serum \( \beta \)-carotene, levels were significantly lower in the single tumor group than in the group with second primary tumors. The authors commented that there were no differences in standard measures of nutritional status and suggested that levels of circulating vitamins A and E may be protective against recurrence of epithelial tumors of the head and neck.

In a study of 22 patients with laryngeal cancer in Poland, Drozdz et al. (1989) found serum retinol and RBP to be lower in cancer patients than in either controls or patients with nonmalignant laryngeal disease. As with several other studies of serum nutrients in patients with diagnosed cancer, it is unclear whether low serum retinol levels were a manifestation of disease or were a pre-existing factor related to cancer risk.

Despite the successes of surgery and radiotherapy in cancers of the head and neck, the failure rate is still high due mainly to the development of secondary primary tumors which are thought to have been in the premalignant state at the time of the first tumor. Thus, treatments that prevent or delay the development of these second primary tumors are of interest. A recent study of Hong et al. (1990) showed that daily administration of high levels of 13-cis-retinoic acid to disease-free patients after primary treatment for squamous carcinomas of the larynx, pharynx, and oral cavity resulted in a significantly lower rate of new second primary tumors in a 12-month period.

Previous epidemiological and case-control studies have demonstrated a direct association between consumption of alcoholic beverages and esophageal cancer (NRC, 1989a). It is likely that smoking acts synergistically with alcohol in this disease (NRC, 1989a). There are substantial international differences in rate (NRC, 1989a). In the U.S., the rate of esophageal cancer is greater among blacks than whites (NRC, 1989a). According to a recent review by Hargreaves et al. (1989), blacks have a poorer nutritional status with respect to a number of nutrients including vitamins A and C. Previous correlational studies have implicated diets low in fresh fruits, vegetables, vitamins A and C, or high in pickled or moldy foods in the etiology of esophageal cancer (NRC, 1989a).
The relationship between dietary factors and esophageal cancer has been addressed in four recent studies. Brown et al. (1988) studied 207 cases and 422 controls among men in a coastal region of South Carolina which is known to have an elevated rate of esophageal cancer. The cases were further divided into an incidence series (identified through referral and hospital admissions and matched to 2 patient controls) and a mortality series (consisting of men who had died of primary esophageal cancer within a 4-year period) which were evaluated separately. The use of alcohol and tobacco and the frequency of intake of 65 selected foods were determined. As may be expected, this study identified alcohol and tobacco use as strong risk factors for esophageal cancer. Increased risk was also associated with a diet low in fresh fruits, particularly citrus fruits and juices. There was no association with fruits high in β-carotene, thus these data were not supportive of an effect of total vitamin A or β-carotene but did suggest that vitamin C may have had protective value.

Li et al. (1989) conducted a case–control study in Linxian, China in which 1244 new cases of esophageal or gastric cancer were matched with community controls. The diet evaluation in this study was difficult to compare to typical studies, but it appeared that consumption of fresh vegetables was associated with an increased risk of esophageal cancer in this population.

Graham et al. (1990) conducted a case–control study in western New York in which 743 cases were identified from hospital records from 1975 to 1986. Of these, usable interviews were obtained from 178 cases (136 men and 42 women) regarding their usual diet 1 year before the start of symptoms; community controls for these cases were matched for age, gender, and neighborhood. In addition to confirming the strong associations of alcohol intake and smoking with esophageal cancer, the results of this study indicated an inverse association between esophageal cancer and intakes of vitamin A (essentially β-carotene) from vegetable sources, with a statistically significant trend for quartiles of intake. Conversely, preformed retinol was associated with esophageal cancer in a direct manner, and this association remained significant after adjustment for smoking and alcohol intake.

The relationship of diet to risk of esophageal cancer was also investigated in a case–control study by Tuyns et al. (1987a) in Calvados, France, a region with a high rate for this type of cancer. The investigators recruited 743 cases (704 males and 39 females) and 1975 age-matched population controls (922 men and 1053 women); however, most of the analysis used only the male controls. A diet–history questionnaire concerning weekly intake of 40 food items was administered by dieticians, and portion sizes were estimated to calculate nutrient values. On average, cases consumed significantly more calories, less protein of animal origin, more carbohydrate and less polyunsaturated fat than controls. The relative risk of esophageal cancer was not significantly associated with the intake of total vitamin A; however, analysis of the sources of vitamin A revealed a sharp contrast between β-carotene which decreased risk (OR=0.53 for the highest quartile) and retinol which increased the relative risk (OR=3.09 for the highest quartile of intake). The high relative risk observed for retinol intake was shown to be due to a higher intakes of organ meats and butter by cases than by controls and was not observed for retinol obtained from other foods (eggs, cheese, and dairy products). A conclusion drawn by the investigators from these results was that "...the role of food items is as important as that of individual nutrients, if not more so."

In summary, the results of these recent studies have been equivocal in implicating dietary intakes of carotene or retinol, or serum levels, as factors associated with esophageal cancer. Some investigations have provided no evidence of significant relationships, while other studies have suggested a protective role for β-carotene but a direct association with high intakes of preformed retinol.
3. Digestive cancer

The rate of stomach cancer in the U.S. has decreased greatly both in incidence and mortality during the past 40 years and is now among the lowest in the world (NRC, 1989b). Previous correlational and case–control studies have provided evidence for an association between consumption of dried, salted, or pickled foods and gastric cancer (NRC, 1989a). In the past 3 years, results of 3 case–control studies relevant to the effects of vitamin A or carotenoids have been reported.

Kono et al. (1983) compared 139 cases of newly diagnosed stomach cancer to both hospital and community controls. The frequency of consumption of foods in fairly broad categories was determined by questionnaire. Compared to hospital controls, cases showed no significant difference or trend with consumption of raw vegetables or green–yellow vegetables or fruits other than mandarin oranges. Compared to the general population controls, there was a significant decrease in relative risk only at the highest frequency of fruit consumption. Thus, this study did not support a role for vitamin A or β–carotene in protection against cancer of the stomach.

You et al. (1988) reported a large case–control study of rural Chinese men and women in which 564 cases were compared to 1131 population controls. Information was collected about 85 food items consumed prior to onset of disease. The odds ratio for stomach cancer decreased with increasing consumption of vegetables and fruits, reaching 0.4 for the highest quartile. Retinol intake showed little or no association with stomach cancer, but there was a trend for carotene across quartiles of intake after adjustment for non–dietary variables. This study generally supported the protective effect of a vegetable–rich diet. The authors noted that intakes of carotene and vitamin C were highly correlated (r=0.6) so that the separate contributions of these nutrients could not be distinguished.

Coggon et al. (1989) conducted a mail–interview study among 95 cases of stomach cancer in England in comparison to population controls. A low intake of "salad vegetables" (mostly lettuce and tomatoes) and a high intake of salt were positively associated with stomach cancer.

The serological study reported by Stähelin et al. (1987), described above under lung cancer, provided evidence that individuals with a low plasma level of vitamins A, C, E, and β–carotene were at greater risk of subsequently developing stomach or large bowel cancers.

Collectively, the data on diet and stomach cancer do not indicate a strong protective effect for vitamin A or β–carotene. The studies are, however, consistent with modest beneficial effects of a diet high in vegetables and fruits.

4. Colorectal cancer

International comparisons have shown a strong association between colorectal cancer and cancers of the breast, endometrium, ovary, and prostate (NRC, 1989a). An association with dietary fat has been demonstrated in some of the case–control and correlational studies (NRC, 1989a). A few previous investigations have shown a relationship between the intake of vitamin A or consumption of certain vegetables (NRC, 1989a; Vogel and McPherson, 1989). The dietary epidemiology of colon cancer has recently been reviewed by Vogel and McPherson (1989) who concluded that "the laboratory and epidemiologic studies to date do not provide supporting evidence for the association of vitamin A–active foods with the risk of colon cancer." Thus, overall, intake of vitamin A and β–carotene does not seem to be closely associated with risk of colorectal cancer (Rogers and Longnecker, 1988; Vogel and McPherson, 1989).
Kune et al. (1987) reported the results of a comparison of 715 cases of colon or rectal cancer and community or hospital controls in Melbourne, Australia. Both food-frequency and food-portion data were collected for 300 foods so that a quantitative assessment of nutrient intake could be made. The relative risk of colorectal cancer was about half in individuals with a diet high in fiber, vegetables, cruciferous vegetables, and vitamin C. Both the reduction in relative risk and the trend by quartiles of intake were significant in a univariate model for both men and women and for both colon and rectal cancers. There was no association of relative risk with retinol intake, but there was an inverse tendency with the use of vitamin A supplements. In a multivariate model, dietary β-carotene had no separate association with the risk of colorectal cancer. This study, which is notable for the quality of dietary information and use of multiple controls, provided evidence that a diet high in fiber and vegetables, including but not limited to those rich in β-carotene, was protective against cancers of the colon and rectum.

La Vecchia et al. (1988) compared 575 cases of colon or rectal cancer in northern Italy with hospital controls. The frequency of nutrient intake was determined from a questionnaire regarding weekly consumption of 29 food items selected to include major sources of retinol and carotenoids. The frequency of intake of green vegetables, tomatoes, and melons was inversely associated with both colon and rectal cancers, and trends across tertiles of intake were significant. Individuals in the highest tertile of carotenoid intake had a relative risk of about 0.6 compared with those in the lowest tertile. No association was found for retinol. The strongest indicator of cancer risk, however, was a combined score based on a high consumption of pasta/rice and beef and a low consumption of green vegetables and coffee.

Tuyns et al. (1987b) also conducted a large case–control study of colorectal cancer among men and women in two cities in Belgium. Eight-hundred eighteen cases were compared to over 2800 population controls. The investigators used a diet history method and food photographs to construct a quantitative measure of retinol and carotenoid intake. Retinol intake was positively associated with colon and rectal cancers, and the trend with quartile of intake was significant. For β-carotene, however, there was no significant trend.

West et al. (1989) examined the relationship of colon cancer in men and women in Utah to dietary intake of macronutrients, dietary fiber, and vitamin A, β-carotene, vitamin C, and cruciferous vegetables. The 4-year study included 231 white male and female cases, ages 40–79 yr, with histologically-confirmed primary colon cancer and 391 population controls matched for age, sex, and address. Questionnaires were administered in respondents' homes by trained interviewers on the average frequency of consumption of 99 foods selected to account for all sources of dietary fiber and over 90 percent of all foods eaten by Utah residents. Quantities consumed were evaluated by use of food models. Nutrient values were calculated and grouped by quartiles for males and females separately.

A high body-mass index was associated with increased risk of colon cancer for both men and women, and dietary fiber was protective in both sexes after adjustment for body mass and energy consumption. Intakes of vitamins A and C did not alter colon cancer risk after adjustment for age, body mass index, crude fiber, and energy intake. However, there was a significant inverse association with the intake of β-carotene for both males and females (adjusted OR of 0.4 and 0.5, respectively, in the highest quartile of intake). A high intake of cruciferous vegetables was also significantly related to reduced risk of colon cancer for men, but not for women. Thus, this study which is notable for quantitative dietary data supports the hypothesis that β-carotene, but not vitamin A, protects against cancer of the colon.

In the prospective study of Californians living in a retirement community by Paganini-Hill et al. (1987), described above under lung cancer, 110 cases of colon cancer were also studied. As was the
case for cancer of the lung, there was no association in either men or women between the level of nutrient intake (either for dietary retinol or β-carotene, or supplemental or total vitamin A) and the rate of colon cancer.

A serological study of French men and women undergoing hospital therapy for cancers of the digestive tract was reported by Charpiot et al. (1989). Little information was provided regarding the patient population or specific types of digestive tract cancers except that hepatocellular carcinomas were excluded, the characteristics of the control group were not described, and no dietary information was collected. The concentrations of retinol, RBP, transthyretin, and α-tocopherol were determined for 50 age-matched, cancer-control pairs. Although each comparison revealed a statistically significant decrease in the cancer group, the simultaneous reduction in serum concentrations of RBP and TTR was interpreted by the authors as an indicator of general malnutrition in the group with cancers of the digestive tract. Thus, this study did not support any preexisting relationship between serum nutrient levels and the subsequent development of digestive tract cancer. In a serological study in Poland, Ostrowski et al. (1987) also found that serum retinol levels were significantly lower in patients with advanced colorectal cancer or metastases. These authors also suggested that these low levels were likely to be a consequence rather than a precursor of the neoplastic process.

Freudenheim et al. (1990a) designed a case-control study in western New York to investigate the relationship of diet to risk of rectal cancer. A series of incident cases (277 Caucasian males and 145 females greater than 40 yr with pathologically confirmed adenocarcinoma of the rectum) were compared to controls matched on age, sex and neighborhood. Detailed interviews by a trained examiner were designed primarily to obtain information on the frequency and approximate quantity of consumption of 129 food items. From these data, indices of intake for energy, fiber, and 15 nutrients were determined.

For males, the risk of rectal cancer increased significantly with increasing intake of total energy, fat, and carbohydrate energy. For females, these relationships were less pronounced but followed the same pattern. For males, there was an increased risk of rectal cancer with increasing intake of preformed vitamin A. However, this relationship was no longer significant after adjustment for either calorie or fat intake. The unadjusted data showed a significant protective effect of carotenoids in males and this trend became stronger after control for calorie or fat intake. Generally, the risk at the highest tertile of carotenoid intake was about half that for the lowest tertile for each level of calorie or fat intake. For females, for whom there were fewer case-control pairs, the tendency for increased risk of rectal cancer with higher retinol consumption and decreased risk with higher carotenoid consumption was also apparent but of lesser magnitude. The data also suggested an inverse association of intakes of vitamin C, and vegetable fiber with risk of rectal cancer, but there was no evidence of protection by vitamin E. The carotenoids, vitamin C and vegetable fiber were highly correlated in food items (r = 0.64 to 0.76 for the male controls). Foods associated with decreased rectal cancer risk included broccoli, celery, lettuce, carrots, green peppers, cucumber, and tomatoes. In all, the results of this study are consistent with protection by diets lower in fat and higher in vegetable fiber, vitamin C, and carotenoids.

Graham et al. (1988) used methodology similar to that in the study by Freudenheim et al. (1990a) to investigate the relationship of total fats, calories, and obesity to risk of colon cancer in the western New York area. Four-hundred twenty-eight cases and 428 matched neighborhood controls were interviewed to obtain data on the intake of individual vegetables, some of which are rich in carotene. Although no significant relationship was found for carotene, vitamin C, and cruciferous vegetables, there were significant reductions in risk for high intakes of tomatoes, peppers, carrots, onions, and celery. Thus, whereas some specific foods were implicated in protection by this study, the overall conclusion regarding a protective effect of β-carotene was, nevertheless, negative.
In summary, several of these recent studies examined a relatively large number of cases and controls, and the quality of dietary information, when obtained, was generally high. While the study of La Vecchia et al. (1988) and particularly those of West et al. (1989) and Freudenheim et al. (1990a) provided some indication for a protective effect of carotenoids, the strongest and most consistent effect was from diets that are high in fiber and vegetables. There was no evidence for any protective effect of dietary retinol. Two clinical intervention trials of the effects of β-carotene on colorectal cancer are currently underway (USDHHS, 1991).

5. **Pancreatic cancer**

The rate of pancreatic cancer in the U.S. has increased over the past 20–30 years, is greater in men than in women, and is somewhat greater for blacks than for whites (NRC, 1989a). Only cigarette smoking has been established as a major risk factor (NRC, 1989a). Most of the previous investigations of the association of fruit and vegetable consumption with pancreatic cancer have reported inverse associations (Rogers and Longecker, 1988). Since 1988, four studies have addressed the relationship of vitamin A to pancreatic cancer.

Falk et al. (1988) conducted a 5-year, hospital-based, case-control study in a high-risk region of southern Louisiana. Three-hundred and sixty-three cases were compared to over 1200 controls. A food-frequency questionnaire was used to collect information about 59 food items; standard portions were assumed to determine intakes of vitamin A, retinol, and β-carotene. Due to the rapid deterioration of patients with pancreatic cancer, more than half of the cases were unavailable for direct interview, in which case dietary information was collected from next of kin. Consumption of fruits was inversely associated with risk of pancreatic cancer, while consumption of vegetables showed a slight, negative association. For β-carotene intake there was no significant association, while for total vitamin A and for retinol, there was a significant positive association at the highest tertile of intake for men but not for women.

The relationship between diet and pancreatic cancer was studied by Farrow and Davis (1990) in a case-control study of married men, aged 20–74 yr, in and around Seattle, Washington. One hundred forty-eight men with newly diagnosed pancreatic cancer were enrolled over a 4-year period. Population controls (n = 188) were matched to cases by 5-year age groups. Information on dietary intake was collected through telephone interviews with wives, even if husbands were available for interview, who subsequently completed a self-administered, semi-quantitative, food-frequency questionnaire concerning intake 3 years prior to diagnosis of foods that represent 96 percent of vitamin A intake, 97 percent of vitamin C intake, and 95 percent of fat intake. In this study, cases tended to have less education than controls, and almost half were current smokers, compared with less than one-fourth of controls. This study found no association between pancreatic cancer risk and the intake of fat or vitamins A or C, although a high intake of protein was implicated as a risk factor.

Burney et al. (1989) have conducted a serological study of 22 cases of pancreatic cancer and 44 controls in western Maryland. Blood was collected in 1974 and stored; cases were collected between 1975 and 1986. These investigators reported no association between subsequent development of cancer of the pancreas and the serum concentration of retinol, β-carotene, total carotenoids, RBP or selenium.

A prospective study of fatal pancreatic cancer in California Seventh-day Adventists was conducted by Mills et al. (1988). Following enrollment in 1976 and completion of a lifestyle questionnaire which included food frequency, there were 40 deaths from pancreatic cancer during 6 years of follow-up. Lower risk was associated with the frequent consumption of vegetarian protein products, beans, lentils and peas, and dried fruits. However, the use of some other items including cooked green vegetables,
green salad, tomatoes, and fresh fruit was not associated with risk of pancreatic cancer. A strength of this study was its prospective nature, including the collection of dietary data well before the onset of disease. However, the lifestyle questionnaire was largely a household questionnaire so that, for some participants, it was filled out by proxy. This study supported the general conclusion that frequent consumption of some vegetables was protective, but it did not lend support to the hypothesis that a diet high in retinol or carotenoids is beneficial.

Thus, none of the dietary studies nor the serological study revealed a consistent or strong association between dietary β-carotene and cancer of the pancreas. The positive relationship between dietary retinol and risk of pancreatic cancer in the Louisiana study may have been due to the more frequent consumption of organ meats by men in this region.

6. Liver cancer

In Western countries, liver cancer is rare (NRC, 1989a). Limited epidemiological evidence has linked alcohol to primary liver cancer. In Africa and parts of Asia, liver cancer is linked to hepatitis B infection and to use of aflatoxin–contaminated foods (NRC, 1989a). No new studies have directly addressed diet and liver cancer.

The study by Kanematsu et al. (1989) examined the associations of serum retinol and liver retinol and of liver retinol and cellular retinol–binding protein (CRBP) in 26 Japanese patients with hepatocellular carcinoma. Serum retinol concentrations were low in these patients. In comparison to normal tissue, carcinomas contained equal concentrations of CRBP, but their retinol content was about one–fifth as great, perhaps reflecting a lower number of vitamin A–storing cells in the cancerous tissue. This study helped to eliminate loss of CRBP as a likely factor in the dedifferentiation of cancerous liver cells. Beta–carotene concentrations have been reported to be lower in cancerous tissue than in adjacent normal tissue at several sites (Palan et al., 1989), but it is unknown whether these differences represent an etiological factor or a consequence of disease.

7. Breast cancer

Breast cancer is a common cause of death among U.S. women, especially Caucasians, is closely correlated with hormonal activity, and in some cases has a genetic predisposition (NRC, 1989a). Nutrients or foods which are considered to be risk factors for breast cancer include fats (NRC, 1989a), total calories, and alcoholic beverages (NRC, 1989a; USDHHS, 1988). Previous studies that have examined a relationship of dietary vitamin A to risk of breast cancer have generally found an inverse association (Rogers and Longnecker, 1988). Serological studies, however, did not show a relationship (Rogers and Longnecker, 1988). Vitamin A and synthetic retinoids have shown strong potential in the chemoprevention of chemically–induced mammary tumors in rodents (Moon, 1989; Moon and Mehta, 1990) and are under clinical study for women at risk of recurrence of breast cancer (Mehta et al., 1991).

Howe et al. (1990) published a review and reanalysis of original data from 12 previously–published, case–control studies conducted in the U.S., Canada, and other countries. Although investigation of the relationship of dietary fat to breast cancer was a major aim of this analysis, information on the intake of vitamin A, β–carotene, retinol, and vitamin C was also available for most of the studies. In all, over 4400 cases and 6000 controls were compared. For post–menopausal women, there was an inverse association between risk and intake of total vitamin A, β–carotene, and vitamin C, but not of retinol. None of these were significant for cases of pre–menopausal breast cancer. The effects were
strongest and statistically significant only for vitamin C. These data are important because of the large experience that these studies represent. It would be desirable to determine whether the effects of β-carotene and vitamin C are independent.

Marubini et al. (1988) investigated 214 cases of breast cancer in Milan, Italy in comparison to hospital controls. Blood was drawn on the first day in the hospital, and a dietary questionnaire with 69 food items was used to calculate an index of dietary retinol and β-carotene. This study reported no association of either dietary retinol or dietary β-carotene and risk of breast cancer. Likewise, plasma β-carotene concentration was not associated with breast cancer. However, a significant direct association between plasma retinol and risk of breast cancer was reported. Because of the well-known effect of disease on plasma nutrients levels, concern may be raised regarding the significance of the plasma data. However, usually disease is associated with decreased plasma nutrient levels and the use of hospital controls should, in principle, have controlled for non-specific effects of illness. Thus, this study provided no evidence for a protective effect of either dietary vitamin A, plasma retinol, or β-carotene on the risk of breast cancer in pre- or postmenopausal women.

van't Veer et al. (1990) conducted a case-control study in The Netherlands of 133 Caucasian women with newly diagnosed breast cancer in comparison to population controls. Interviews were conducted by trained dieticians using a questionnaire that included 236 foods; portion sizes were also estimated to provide a quantitative assessment of nutrient intake. The levels of consumption of dietary fiber, cereal products and total vegetable products were associated with a somewhat lower risk of breast cancer although the trend was significant only for cereals. There was a significant correlation between dietary β-carotene intake and plasma levels of β-carotene and carotenoids, thus providing a form of validation for the dietary data. For β-carotene consumption, the relative risk of breast cancer did not differ significantly from 1 except at the highest quartile of intake (relative risk = 0.63). The trend across quartiles, however, was not significant. Although the results generally supported a protective effect of a diet rich in vegetable products, the data did not indicate that β-carotene content was likely to be responsible.

A case-control study conducted in south Australia by Rohan et al. (1988) investigated the effect of diet on breast cancer in 451 cases and an equal number of community controls. A self-administered, food-frequency questionnaire was used to establish quintiles of intake for retinol and β-carotene. For retinol, there was no association with risk of breast cancer for either pre- or postmenopausal women. For β-carotene, the relative risk for all cases for each of the upper 2 quintiles of intake was 0.76 and the linear trend was significant. When pre- and postmenopausal women were considered separately, the trends were no longer significant. Overall, this study provided modest support for a protective role of dietary β-carotene, but not of retinol, in reducing the risk of breast cancer.

This same groups of investigators (Rohan et al., 1990) also conducted a case-control study of the effect of diet on benign proliferative epithelial disorders (BPED) in the same population group. Three-hundred eighty-three cases with a biopsy indicating BPED were compared to 192 controls whose biopsy did not show proliferation and to 383 community controls matched on the basis of age and socioeconomic grading. An interviewer-administered questionnaire was used to collect sociodemographic and medical information, and a self-administered, quantitative food-frequency questionnaire was used to ascertain total daily intake of energy and selected nutrients.

For retinol intake, there was a significant trend (inverse relationship) with risk of BPED when cases were compared to community controls but not to biopsy controls. The same pattern was observed for β-carotene intake, as well as for dietary fiber. These authors suggested that these discrepancies in outcome may have been due to the problem of "overmatching" of cases and community controls, i.e., that one or more of the characteristics used for matching may have been a crude proxy for dietary habits (Rohan et al., 1990). Trends between relative risk of BPED and quintile of intake of total and

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saturated fat, after adjustment for energy intake, were not statistically significant when cases were compared to either control group. Thus, this study did not support a role for fat intake in the etiology of breast cancer but provided some suggestion of an inverse association with the intake of retinol, β-carotene, and fiber.

Ewertz and Gill (1990) conducted a large case-control study among Danish women, aged less than 70 yr, which was principally designed to assess the relationship between dietary fat and breast cancer risk. Diet information was collected by a self-administered, semi-quantitative, food-frequency questionnaire that was mailed 1 year after diagnosis of breast cancer to avoid the period of adjuvant chemotherapy. This questionnaire included 21 food items which cover about 80 percent of the consumption of fat and β-carotene and questions about use of supplements, coffee, tea, sugar, and artificial sweeteners. While there was a highly significant trend for increased risk with increasing intake of fat, the results did not support a relationship between consumption of β-carotene from vegetable or non-vegetable sources and breast cancer risk.

Brisson et al. (1989) conducted a case-control study of 290 women with newly diagnosed breast cancer and 645 population controls. This study differed in design from most case-control studies in that the cases were used to establish mammographic characteristics associated with breast cancer. The mammographic characteristics of the control group were then determined, and this group was divided into women with high-risk or low-risk mammographic characteristics for breast cancer. The dietary component of the study was then conducted on the subgroups of the control women. This experimental design assured that diet was not confounded by disease. A greater extent of high-risk mammographic features was found in women with a high intake of saturated fat, while increasing carotenoid or fiber intake was significantly associated with a reduction in high-risk mammographic features. Retinol had little effect. The authors suggested that an elevation of dietary saturated fat and a reduction of carotenoids and fiber may increase breast cancer risk through effects on breast tissue morphology.

Hislop et al. (1990) reported results of a study of women in Vancouver, Canada, ages 40–59, with benign breast disease classified by two standard histological criteria into proliferative lesions associated with a high risk of breast cancer and lesions causing no increased risk. Dietary data were obtained from a self-administered questionnaire and consisted of the usual frequency of intake during the past year of 39 food items selected for their fat, provitamin A, and vitamin A content. From over 7000 women who initially took part in a breast screening project, there were 124 and 274 case-control pairs in the increased risk group and no increased risk group using one criterion (proliferative lesions showing moderate or severe epithelial hyperplasia regardless of atypia classified as at increased breast cancer risk), and somewhat fewer cases and controls in the increased-risk group when a second, more stringent, histological criterion (ductal and lobular hyperplastic lesions with severe atypia classified as at-increased-risk) was applied.

Using criterion 1, a significantly greater number of women classified as at-increased-risk of breast cancer were non-users of vitamin A supplements, although quantitative measures of use were not examined. Consumption of green vegetables was marginally significant, but intakes of yellow vegetables, carrots, and fruit or animal sources of vitamin A were not. Using criterion 2, neither the use of vitamin A supplements nor intake of any of the foods rich in vitamin A or β-carotene was associated with a significant difference in risk. Thus, the results of this study only weakly support the hypothesis that vitamin A is protective against breast changes associated with increased cancer risk.

Katsouyanni et al. (1988) compared 120 women with newly diagnosed breast cancer to 120 patients admitted for trauma or orthopedic conditions in an Athens, Greece hospital. Interviews were conducted in the hospital to determine the typical use of 120 food items. Cases reported significantly less frequent consumption of total vitamin A, with a relative risk ratio of 0.46 after adjustment for
potentially confounding factors. When analyzed by retinol and β-carotene content, the inverse associations were similar, but the significance of each was less than for total vitamin A. Overall, this study provided support for a protective role of foods containing dietary vitamin A.

In a 2-year study of women in northern Italy with histologically diagnosed breast adenocarcinoma who were free of local or distant metastases, Toniolo et al. (1989) used a semi-quantitative, food-frequency questionnaire to collect data on intake of 70 food items from which the daily intake of β-carotene, retinol, vitamin C, vitamin E, and fat were calculated. Data were analyzed for 251 cases and 499 age-matched population controls. The intake of β-carotene was slightly higher among cases than controls while that of retinol was slightly lower, but these differences were not significant. There were no apparent differences in intake of vitamins C and E. Although this study supported the concept that a low fat intake is protective against breast cancer, it provided no evidence in support of either β-carotene or vitamin A, or of intake of vegetables or fruits, as protective factors.

A one-year, case-control study was conducted by Potischman et al. (1990) in women in western New York who were being evaluated for a breast mass but who had no previous history of cancer. Prior to biopsy, a fasting blood sample was collected and a self-administered dietary questionnaire of 30 food items (selected to account for 90 percent of vitamin A) intake was completed. Women were classified according to their pathology report into those with breast cancer (n = 83), controls (n = 113, including 34 without biopsy), and those with high-risk atypical hyperplasia (n = 40) who were excluded from the study. Cancer cases were somewhat older than controls (mean ages 58 and 50 yr, respectively). The mean dietary intakes of total vitamin A and vitamin A from vegetable sources were not significantly different. The plasma levels of retinol were identical between cases and controls. However, the plasma levels of β-carotene and lycopene, but not α-carotene, were significantly lower in cases than controls. The trend with quartile of plasma nutrients was significant for β-carotene both before and after adjustment for plasma lipid concentration and, on further analysis, was restricted to postmenopausal cases. There was no correlation between dietary vitamin A calculated from vegetable sources and plasma β-carotene (r = –0.02), but the food-frequency instrument used in this study was acknowledged to be relatively weak. In all, the study did not point to a relationship between dietary vitamin A or β-carotene and breast cancer but did suggest a possible relationship between plasma β-carotene and this disease.

In a retrospective serological study, Basu et al. (1989) found no statistically significant differences in the concentrations of serum vitamin A, β-carotene, vitamin E, or selenium in 30 women with advanced breast cancer whose stored sera were compared to sera from 30 women with benign breast disease and 30 healthy, age-matched controls. Although the mean values for vitamin A and RBP were somewhat lower in women with breast cancer than in the control group, they were also lower in the group with benign breast disease; only in the case of serum RBP were differences significant between the women with breast cancer and the healthy controls. This report lacked information on the year of the study, the time since sample collection, characteristics of the study population excepting age, and it did not address dietary intake. The authors concluded that the somewhat lower values for serum vitamin A may well have been a consequence of disease in general rather than breast cancer per se.

In the prospective dietary study of Paganini-Hill et al. (1987), described above, 123 cases of breast cancer occurred in women in a California retirement community. The consumption of dietary vitamin A or β-carotene as well as supplemental vitamin A was determined by a quantitative food-frequency questionnaire. As was the case for lung and colon cancers, there was no evidence that more frequent use of any of these forms of vitamin A resulted in protection from breast cancer.

The prospective serological study of Knekt et al. (1990) in Finnish men and women (see under Lung Cancer) provided evidence for an association of low serum β-carotene, but not retinol, and subsequent
risk of breast cancer. Of cancers at all sites, this was the only statistically significant association for females.

In summary, the results of several dietary studies on vitamin A and human breast cancer are inconclusive. None of the recent or previous reports provided strong evidence of an inverse association, some studies were consistent with a very modest beneficial effect, while in others no measurable protection was afforded. None of the ongoing clinical trials listed in the Surgeon General's report (USDHHS, 1988) has been designed to address the effects of vitamin A on breast cancer.

8. Cervical/ovarian cancer

In the U.S., ovarian cancer is the major cause of death from cancer of the reproductive tract in women. No clear dietary association has been reported (NRC, 1989a). In an earlier study, a very small direct association was seen between vitamin A intake and the risk of ovarian cancer. Two recent case-control studies have examined dietary factors associated with ovarian cancer.

Slattery et al. (1989) compared 35 cases with first primary ovarian cancer to 492 population controls in Utah. Information was obtained by interview on the frequency and quantity of consumption of 183 common food items. This study did not find evidence of association between calories, protein, or fat and risk of ovarian cancer. For intake of vitamins A and C there was a slight but non-significant inverse association with risk. For β-carotene, the inverse association was statistically significant. Women in the highest tertile of β-carotene intake consumed about twice as much β-carotene and had half the risk of ovarian cancer as compared to women in the lowest tertile of β-carotene intake.

In a case-control study in China (Shu et al., 1989), 172 cases were compared to 172 community controls. Information was obtained on the consumption of 63 common foods. This study identified calories and fat from animal sources as positive risk factors. There was no association between risk of ovarian cancer and either vitamin A or carotene in foods. There was no evidence that frequent consumption of dark green, yellow-orange vegetables, or cruciferous vegetables conferred protection from ovarian cancer.

In summary, no consistent pattern regarding a protective effect of either retinol or β-carotene has emerged from the few dietary studies that have examined ovarian cancer. The collection of dietary data in the study of Slattery et al. (1989) was especially thorough, and the results of this study suggested a modest, beneficial effect of β-carotene-rich foods.

The relationship of diet to cervical cancer was not addressed in the benchmark references (NRC, 1989a; USDHHS, 1988). Cervical cancer is, like lung cancer, primarily a squamous cell epithelial tumor. Its prevalence is greater among women of low socioeconomic status and thus may be related to poor nutrition (Ziegler, 1991). Epidemiological evidence for the role of vitamins in the etiology of cervical neoplasia was reviewed by Schneider and Shah (1989). Of six case-control studies including dietary analysis that were reported previously, two found no association between vitamin A and cervical cancer risk, while four reported a significant relationship between low β-carotene intake and risk (Schneider and Shah, 1989). Five case-control studies and one prospective clinical trial have been published recently.

A case-control study by Brock et al. (1988) in Australia compared the plasma β-carotene concentrations and quantitative nutrient intakes for 117 cases with cervical carcinoma in situ and 196 community controls. These authors reported no significant association between dietary retinol or β-carotene and risk of cervical carcinoma in situ. There was also no relationship with plasma retinol. However, the plasma concentration of β-carotene was significantly lower for cases than for controls.
Since plasma β-carotene is thought to reflect dietary intake, the lack of association with dietary β-carotene in the face of a significant difference in plasma β-carotene was puzzling. Although it is possible that the lower plasma β-carotene concentrations were related to incipient disease, the authors commented that carcinoma in situ is an early form of cancer that is not known to be associated with changes in appetite and metabolism that could affect blood nutrient concentrations.

Verreault et al. (1989) compared 189 women in Seattle with a diagnosis of cervical cancer to 227 community controls. Dietary habits during the year preceding diagnosis were determined by a telephone interview–questionnaire. Although cases and controls were matched for age, the case group tended to have somewhat less education, more smokers, and a history of more sexual activity. Regarding diet, retinol intake showed no relationship to risk of cervical cancer. Frequent consumption of dark green, leafy vegetables was related to a lower risk. After adjustment for energy intake and known risk factors including smoking, the inverse relationship between dietary β-carotene and risk of cervical cancer remained, but it was not statistically significant. An inverse relationship was also found for fruit juice; this association remained significant after adjustment. The results of this study were consistent with a protective effect of β-carotene, but the effect was not strong. The stronger association seen with fruit juice might suggest that the vitamin C in foods that are also rich in β-carotene was responsible for some of the protective effect.

De Vet and colleagues (1991) have recently reported the results of a randomized, blind, multi-center clinical trial of the efficacy of supplemental β-carotene to retard progression or cause regression of cervical dysplasia, a condition thought to be a precursor to cervical cancer. Two-hundred eighty-one women, ages 20–65, with a recent diagnosis of cervical dysplasia were stratified by age, location, and degree of dysplasia; 137 were assigned to receive 10 mg of β-carotene daily for 3 months. Habitual dietary intake was determined by a mid-study questionnaire. There was insufficient progression of dysplasia to be analyzed. Regarding regression, there were no statistically significant differences between the experimental and the control groups. The authors considered that the time of study may have been too short, or that the typical Dutch diet may be sufficient in β-carotene to mask a small effect of additional β-carotene. By comparison, it may be recalled that while histologic improvement in oral leukoplakia (reduction in cells with micronuclei) was observed after 3 months of β-carotene supplementation at a dose of 180 mg per week, regression was evident only after 6 months (Stich et al., 1988); thus, both the time of treatment and the dose of β-carotene may have been too little for differences in cervical dysplasia to become manifest.

A case–control study of invasive cervical cancer among women in 5 geographically diverse areas of the U.S. was reported recently (Ziegler, 1991; Ziegler et al., 1990). Data were reported for white women with whom an interview could be obtained (271 cases equalling 73 percent of white, non–Hispanic cases and 502 controls equalling 74 percent of identified controls). Diet was assessed by asking about the usual frequency of consumption of 75 food items that included the major sources of carotenoids, vitamin A, vitamin C, and folate in the diets of U.S. women. The weekly frequency of consumption was derived for each food item; typical portions were assumed. Ten cases and 4 controls were excluded due to incomplete data for 6 or more food items. Cervical cancer diagnoses (histological classification and stage) were based on hospital records: of 261 cases, 218 were squamous cancers, 14 adenosquamous cancers, and 29 adenocarcinomas.

Analysis of the relative risks of invasive squamous cell cervical cancer, by either quartiles or quintiles of intake of carotenoids, vitamin A, vitamin C, or folate, did not reveal significant trends. Similar results were found for both crude relative risks and after adjustment for factors related to cervical cancer risk. Analysis by food groups (including vegetables and fruit, dark green vegetables, and dark yellow–orange vegetables) also did not reveal significant trends, nor were there trends with the use of multivitamin supplements nor with specific vitamins including vitamin A in supplements. Thus, the findings of this study of U.S. women did not implicate consumption of vitamin A or other
antioxidant vitamins as protective for invasive squamous cell cervical carcinoma. The authors noted that their results differ from those of other investigations with comparable cases and controls in which intake of at least one micronutrient was lower in women with cervical disease (dysplasia, carcinoma, or invasive cervical cancer) and that the results are not readily explained by bias in subject selection, inadequate power, or lack of control of confounding factors.

Palan et al. (1988) reported that the serum β-carotene concentrations, but not those of retinol, were significantly lower in women with newly diagnosed mild or moderate dysplasia, severe dysplasia or carcinoma in situ, or cancer of the cervix than in disease-free controls. There was a stepwise, progressive decline in β-carotene concentration with increased severity of disease. As with other studies of this type, it is unclear whether the low serum β-carotene concentrations are a manifestation of disease or reflect risk factors related to disease. The authors commented that patients with cervical dysplasia are asymptomatic, and the low plasma β-carotene levels may reflect differences in dietary intake, as shown in earlier work.

Cuzick et al. (1990) reported results of a case-control study of London women, aged 16-40 yr, with histologically classified cervical intraepithelial neoplasia and community controls. Blood was collected from 86 percent of the cases and 68 percent of the randomly selected controls; serum was analyzed for vitamins A and E. No dietary information was reported. There were no significant differences for vitamin A in either the means, odds ratios, or trends with quintile of serum vitamin A. Significant trends were observed for vitamin E for both classifications of neoplasia examined, with higher levels of vitamin E being protective. Thus, this study did not reveal any relationship between serum vitamin A and cervical neoplasia, even in women with diagnosed disease at the time of blood sampling.

Overall, the results of the recent studies of diet and cervical cancer provide very little, if any, support for a protective role of β-carotene–rich foods. The results of the short-term intervention trial (de Vet et al., 1991) did not support a therapeutic effect for supplemental β-carotene in cervical dysplasia. The results of serological studies may be of etiologic significance, but they could also reflect occult disease; further long-term prospective studies would be needed to address these data.

9. **Prostate cancer**

Cancer of the prostate is common in the U.S.. The incidence increases with age after about 45 yr of age (NRC, 1989a). The rate for black men is greater than for white men and is increasing (Hargreaves, 1989; NRC, 1989a). Male hormones and sexual activity appear to contribute to risk (NRC, 1989a). A number of dietary studies have identified fats and vitamin A as probable risk factors (NRC, 1989a). For prostate cancer, particularly in older men, some of the previous studies had identified retinol as a positive risk factor. Some studies had suggested a protective effect for dietary β-carotene (NRC, 1989a; Rogers and Longnecker, 1988).

Hsing et al. (1990a) reported a case-control serological study with 103 men in western Maryland who developed prostate cancer, in comparison to matched community controls. This study examined serum nutrient concentrations after a long period of blood storage which is likely to have led to deterioration of some nutrients. For serum retinol, there was a small downward shift in the distribution for cases (mean 61 μg/dl) vs controls (mean 64 μg/dl). There was a tendency for greater risk of prostate cancer to be associated with a lower serum retinol. For β-carotene, the opposite tendency was observed. In all, this study provided little support for reduced risk in men with either a high concentration of serum retinol or β-carotene.

A study conducted in the Netherlands by Hayes et al. (1988) examined both plasma retinol and β-carotene levels in 94 cases of clinical prostate cancer in comparison to 130 men with benign prostatic
hyperplasia (BPH) and 130 hospital controls. Men with prostate cancer had lower mean retinol concentrations and lower mean \( \beta \)-carotene and \( \alpha \)-tocopherol concentrations than the controls. Some of this difference may have been related to disease because plasma nutrient levels tended to rise slightly after surgical treatment. At face value, the results indicated that a low plasma retinol concentration was associated with increased risk of prostate cancer.

Two publications (Ohno et al., 1988; Oishi et al., 1988) have reported on a group of 100 men with newly diagnosed prostate cancer in Japan who were compared to 100 men with benign prostatic disease and 100 hospital controls. Beta-carotene intake was significantly lower in prostate cancer patients than in either men with BPH or hospital controls. When \( \beta \)-carotene intake was examined by quartiles for all ages, the relative risk of prostate cancer in the lowest quartile for intake was about three times greater than that in the upper quartile; this difference was highly significant. When effects were examined by age, a diet high in \( \beta \)-carotene appeared to confer protection in older men (\( \geq 70 \) yr) but not in younger men. Thus, the results of this study indicated a modest protective effect of dietary \( \beta \)-carotene for cancer of the prostate.

Mettlin et al. (1989) examined the dietary habits of 371 men with newly diagnosed prostate cancer in comparison with hospital controls. A food–frequency checklist was completed shortly before hospital admission and standard food portions were assumed. For men <68 yr, the relative risk was lower in men who consumed more \( \beta \)-carotene. However, for older men this difference was not apparent. For the entire study, the evidence was consistent with some reduction of risk of prostate cancer with higher consumption of \( \beta \)-carotene.

Ross et al. (1987) conducted a case–control study of black men and white men in a region of southern California with a high rate of prostate cancer among blacks. One-hundred forty-two cases were matched with population controls. Among dietary variables, a high intake of fat was a risk factor for both black men and white men. There was no significant or consistent association of dietary vitamin A or \( \beta \)-carotene with prostate cancer.

Kolonel et al. (1988) compared 452 men with prostate cancer in Hawaii with 899 population controls. Data were analyzed by age and ethnic group. Collection of dietary data in this study was very thorough so that a quantitative assessment of nutrient intake could be calculated. This study found a positive association between the intake of total vitamin A, carotenoids, and \( \beta \)-carotene and prostate cancer in older men (\( \geq 70 \) yr), but not in younger men. The overall trend was significant. For dietary retinol, however, there was no significant relationship with prostate cancer. It is of interest that the same diet methodology had been used previously in this population in a study of lung cancer (Le Marchand et al., 1989) and an inverse relationship had been observed between total vitamin A or \( \beta \)-carotene intake and cancer of the lung.

A re-evaluation of these data on diet and prostate cancer were reported in 1991 (Le Marchand et al.). Data were further analyzed by food groups rich in \( \beta \)-carotene. Le Marchand et al. (1991) concluded that the greater \( \beta \)-carotene intake in controls vs cases was due to more frequent consumption of papaya. However, there was no association between risk of prostate cancer and consumption of other \( \beta \)-carotene-rich foods. Thus, they concluded that the intake of \( \beta \)-carotene, lycopene, lutein, or other phytochemicals was not associated with prostate cancer risk.

A prospective study was reported by Reichman et al. (1990) of a cohort of 2440 men who participated in the NHANES I Epidemiologic Follow-up Study. Eighty-four cases of prostate cancer developed during a 10-year period. Data for initial serum vitamin A concentration was compared between cases and the remaining group. For the entire population, serum vitamin A level was itself positively correlated with education, serum cholesterol, alcohol consumption and, to a lesser extent, with body-mass index. A significant difference was found between the mean serum vitamin A concentration for
cases (59 μg/dl) vs non-cases (65 μg/dl). When considered by quartiles of serum vitamin A, there were significantly fewer cases whose initial vitamin A level had been high. While it is possible that some of the men who developed clinically-recognized cancer had incipient disease at the time of the initial blood sampling, the authors did not think this was likely to have confounded the results because there was no relationship between the time interval between blood sampling and diagnosis and the initial serum vitamin A level. Thus, this study indicated that a low serum retinol concentration could be a risk factor for cancer of the prostate.

Another prospective study was reported by Hsing et al. (1990b) of a cohort of over 17,500 white males who were part of the Lutheran Brotherhood Study. During a 20-year period, 149 died of prostate cancer. Information on the frequency of consumption of 35 food items, grouped in 9 food groups, was collected in 1966. However, a number of potentially important sources of vitamin A (such as liver, cheese, butter, broccoli, spinach, and cantaloupe) were not included in the questionnaire. This study found no significant association between frequency of intake of any of the 9 food groups (including vegetables, cruciferous vegetables, and fruits) and the relative risk of prostate cancer. When analyzed by retinol and β-carotene content, risk was directly related to the intake of total vitamin A, retinol, and β-carotene for men <75 yr, while risk decreased significantly with total vitamin A and β-carotene for older men. Thus, this study suggested that there may be age-specific differences in diet-risk relationships for prostate cancer. However, other authors have noted that there is no biological hypothesis regarding differences between age groups for this disease (Mettlin et al., 1989).

A third prospective study was reported by Severson et al. (1989) for a cohort of nearly 8000 men of Japanese ancestry living in Hawaii. One-hundred seventy-four cases of prostate cancer were compared to the remaining non-case controls. A 24-hour dietary recall interview with food models was used to collect dietary data. The emphasis of this study was on food items typical of the Japanese vs Western diet and there was no analysis of vitamin A or β-carotene per se. The risk of prostate cancer appeared to be lower in men who consumed more rice and tofu, and there was a marginal, positive relationship between cancer risk and consumption of butter and eggs. Thus, the relationship of prostate cancer to some aspects of the diet was strengthened by this study, but the results provided little direct information concerning vitamin A.

In the prospective study of Paganini-Hill et al. (1987), 93 cases of prostate cancer developed. As was the case for the other cancer sites examined in this study, there was no relationship between intake of total vitamin A, carotenoids, retinol, or β-carotene and subsequent development of prostate cancer.

In contrast, the prospective serological study of Finnish men and women by Knekt et al. (1990) (see Lung cancer) provided evidence of an association of low baseline serum β-carotene, but not retinol, with the development of prostate cancer during an 8-year follow-up period.

In all, the data relating vitamin A and carotene consumption, or plasma nutrient levels, to risk of prostate cancer continue to be inconsistent. Some of the previous investigations and some of the more recent studies have found a protective effect for retinol or carotenoids, while others have found no effect or even a positive association.

10. **Bladder cancer**

Bladder cancer is more common in the U.S. than in many other parts of the world and is more frequent in men than in women (NRC, 1989a). Bladder cancer is associated with cigarette smoking and certain occupational hazards (NRC, 1989a). A few previous dietary studies have suggested either a protective effect of vitamin A and β-carotene or no effect (NRC, 1989a). Synthetic retinoids have been effective in the prevention of experimental bladder cancer in animals (NRC, 1989a), and one of
these retinoids, etretinate, has showed efficacy in clinical trials of prevention of recurrent superficial bladder cancer (reviewed in Lippman and Meyskens, 1988). Four recent epidemiological studies have reported on the relationship of diet or serological measurements and bladder cancer.

La Vecchia et al. (1989) reported a case–control study of 163 histologically confirmed cases of bladder cancer vs 181 hospital controls in northern Italy. A questionnaire was used to determine the frequency of consumption, before illness, of foods selected to include major sources of retinoids and carotenoids. This study reported that the intake of green vegetables and carrots was significantly lower for cases, as was intake of total carotenoids and total vitamin A. For retinol intake, there was no significant difference.

In the prospective study of Paganini–Hill et al. (1987), no relationship between dietary intake of total vitamin A, retinol, carotenoids, or β–carotene was found for 59 men and women who subsequently developed cancer of the bladder. However, it is of interest that these authors subsequently analyzed their data, for all cancer sites combined, by histological type into five categories: adenocarcinomas, transitional cell, epithelial cell, squamous cell, and all others (Paganini–Hill et al., 1987). Data were then analyzed by tertile of dietary vitamin A or β–carotene intake and by supplement use. For tumors with transitional cell histology, but not for any other classification, there was a significant, inverse trend for β–carotene intake and a nearly significant inverse trend for vitamin A intake. There was no significant trend for supplements. The 53 transitional cell cancers included 45 bladder cancers. Thus, this follow-up analysis of all cancer sites may be interpreted as suggesting a beneficial effect of carotene intake on the risk of certain types of bladder cancer.

In a prospective serological study conducted in western Maryland, serum concentrations of retinol, RBP, β–carotene, and lycopene were compared for 35 cases of bladder cancer vs matched controls. There were no significant differences between bladder cancer cases and population controls for any of these measurements (Helzlsouer et al., 1989).

A study of the relationship of urothelial cancer to dietary habits and vitamin supplement use among men and women in Stockholm, Sweden was reported by Steineck et al. (1990). Data were analyzed for 323 cases less than 74 yr who had histologically or cytologically confirmed urothelial cancer and/or squamous cell cancer of the lower urinary tract (renal pelvis, ureter, urinary bladder and urethra, of which 305 cases were of bladder cancer) and for 392 population controls. Dietary information was obtained of 56 food items chosen primarily to cover the major contributors of carotene, retinol, vitamin C, and fried foods in the Swedish diet. Information about smoking habits and the frequency of use of supplements mainly containing vitamins A, B, and C was also obtained. This study reported a strong inverse association between use of supplements mainly containing vitamin A and the risk of urothelial cancer; however, this conclusion was based on use of such supplements by none of the cases and only eight of the controls. Additionally, nearly all the subjects exposed to vitamin A supplements were also exposed to vitamin C supplements. Regarding dietary vitamins, there were no significant associations with β–carotene, retinol, or vitamin C intake and risk of urothelial cancer.

In summary, the number of human studies regarding diet and bladder cancer is still quite small, and consistent effects for dietary vitamin A or carotenoids have not yet emerged.

11. Skin cancer

Skin cancer was not considered specifically in the benchmark reports. Synthetic retinoids have become a major form of chemotherapy for a number of previously intractable dermatological disorders (Bollag, 1983; Lippman and Meyskens, 1988), and high-dose β–carotene has been used successfully for a
number of years in the treatment of the photosensitive disease, erythropoietic protoporphyria (Matthews–Roth, 1989). In animal models of chemically–induced photosensitization, both high–dose β–carotene and a structurally–similar carotenoid without provitamin A activity were effective, pointing to a mechanism not involving retinol (Mathews–Roth, 1989). Since 1987, a case–control study and a major clinical trial have addressed the ability of vitamin A or β–carotene from the diet, or supplemental β–carotene, to prevent cancer of the skin.

A case–control study of malignant melanoma was conducted by Stryker et al. (1990) among patients at a Massachusetts dermatology clinic. Two–hundred four cases were compared to 248 controls with other dermatological conditions. A semiquantitative food–frequency questionnaire was used and plasma was collected at the first visit for analysis of retinol, β–carotene, α–carotene, lycopene and α–tocopherol. For plasma α– or β–carotene, there were no appreciable differences between cases and controls. The intake of preformed vitamin A was similar in both groups. Controls had a tendency to consume more β–carotene, but there were no significant associations with specific β–carotene–rich foods. Thus, the overall result of this study was that neither diet nor blood levels of retinol nor carotenoids were associated with risk of malignant melanoma.

The results of a major, prospective, clinical trial on the efficacy of supplemental β–carotene to prevent basal–cell and squamous–cell cancers of the skin was reported by Greenberg and colleagues (1990). The authors noted that patients who have recently had a non–melanoma skin cancer are at high risk for another skin cancer and thus this group seemed well–suited for a clinical prevention trial. Over 1800 patients at 4 medical centers who had a recent non–melanoma skin cancer were randomly assigned to either the treatment group (50 mg β–carotene/d by capsule) or the control group (placebo capsule). These groups were well matched at the study's outset. All subjects underwent an annual dermatological examination and plasma was analyzed at enrollment and annually thereafter for retinol and β–carotene. A limited dietary questionnaire was administered at intervals throughout the study. The primary end point was the first occurrence of a new basal–cell or squamous–cell skin cancer. Nearly all lesions were confirmed histologically.

After five years, the study was halted and results were thoroughly analyzed. The risk of a new skin cancer in the β–carotene–supplemented group and the placebo group was essentially identical. Subgroup analysis indicated that β–carotene did not lower the risk of skin cancer for patients who smoked or for those whose plasma β–carotene concentration was low at enrollment. Compliance appeared to have been good. It is noteworthy that the plasma β–carotene concentration was 8–fold greater in the β–carotene–supplemented group than in the placebo group. Since not all subjects completed five years of treatment, it was important to analyze whether protection could be observed in those treated with β–carotene for longer times. The results showed no evidence that efficacy increased with the length of β–carotene treatment. Thus, this carefully designed and conducted study did not provide evidence that supplemental β–carotene protects against recurring cancers of the skin.

It has been commented that the latency for skin cancer is long such that the efficacy of supplemental β–carotene in preventing primary skin cancers still should be subjected to long–term follow–up (Manson et al., 1991).
IV. SUMMARY AND CONCLUSIONS

A. VITAMIN A: NORMAL METABOLISM AND PHYSIOLOGY

It is now recognized that vitamin A functions in multiple physiological processes. Outside of its critical function in vision, retinol or one of its metabolites is required for normal cell differentiation, growth, and reproduction. It is possible that the role of vitamin A in each of these processes is associated with more than one type of biochemical, regulatory event. As more has been learned about the processes in which retinol functions, the physiological function(s) of retinol or its metabolite, retinoic acid, appear to be more like those of the hormones that govern a wide variety of biological processes and which frequently interact to maintain homeostasis. The recent recognition of nuclear receptors for retinoic acid, described in Section II. C., has further strengthened the analogy between vitamin A and the steroid hormones. Despite this improved understanding, it is still unknown exactly how retinol or related retinoids regulate differentiation, or whether any of these processes is related directly to preventing the type of unregulated cell growth that characterizes cancer.

B. BASIS OF THE ASSOCIATION BETWEEN VITAMIN A AND CANCER

An important question to be addressed in any diet–cancer relationship is whether cancer risk is associated with a specific nutrient (e.g., retinol, β-carotene, carotenoids, or other nutrients), with a particular food(s), or with some combination of factors. The recent human studies on vitamin A and cancer summarized above support most strongly an inverse relationship between cancer risk and the frequent intake of certain foods. The most consistent protective effect was seen with diets high in vegetables and/or fruits. Significant differences or tendencies consistent with a beneficial effect of such diets were seen in studies of cancer of several sites: the lung (Bond et al., 1987; Connell et al., 1989; Dartigues et al., 1990; Fontham et al., 1988; Jain et al., 1990; Koo, 1988; Le Marchand et al., 1989), mouth and pharynx (Franco et al., 1989; McLaughlin et al., 1988), esophagus (Brown et al., 1988; Graham et al., 1990), stomach (You et al., 1988), colon/rectum (Freudenberg et al., 1989, 1990; Kune et al., 1987; La Vecchia et al., 1988; Tuyns et al., 1987b; West et al., 1989), pancreas (Falk et al., 1988; Mills et al., 1988), breast (Brisson et al., 1989; Howe et al., 1990; Katsouyani et al., 1988; Rohan et al., 1989, 1990; van’t Veer et al., 1990), ovaries (Slattery et al., 1989), cervix (Verreault et al., 1989), prostate (Mettlin et al., 1989; Ohno et al., 1988; Oishi et al., 1988), and bladder (La Vecchia et al., 1989).

As stated by Miccozzi (1989), "People consume (and epidemiological studies inquire about) foods, culturally processed in different ways, as opposed to nutrients." The primary data in all of the dietary studies above were the frequency of use or the quantity of intake of food items or groups of foods. Thereafter, data on the consumption of nutrients such as retinol or β-carotene were derived by calculation. These data on nutrient intake thus rest on various assumptions about quantities of foods actually consumed as well as their nutrient content. A protective effect of β-carotene or retinol could be inferred from some studies but not from others. If the association between the intake of foods rich in β-carotene or retinol and cancer risk was consistently strong and if correlations with other nutrients were not apparent, then it would be possible to argue that the beneficial effect of these foods is highly likely (though still not proven) to be due to their contents of vitamin A.

In a number of the studies cited above, cancer risk was associated with consumption of certain provitamin A–rich foods but not with others, or the protective effect of fruits and vegetables did not appear to be closely related to their provitamin A content. Possible explanations for these results include: 1) that effects are due to other factors which are found in some provitamin A–rich foods; 2)
that the effects are largely due to vitamin A but vary with its food source, i.e., that there are significant interactions with other factors in foods; 3) that effects of provitamin A are seen primarily in combination(s) with other factors that are found in some but not all vitamin A–rich foods; or 4) that some of the data on nutrient content may be inaccurate and unreliable. In any case, a protective effect of provitamin A (β-carotene) has been observed less consistently than that of a diet high in fruits and vegetables. It therefore seems prudent to conclude that the weight of evidence supports a beneficial effect of foods or food combinations more strongly than it supports any specific benefit from dietary β-carotene or retinol. This conclusion is similar to that reached recently by other reviewers who have examined the evidence relating vitamin A to lung cancer (Colditz et al., 1987; Willett, 1990) or to cancers more generally (Freudenheim and Graham, 1989; Ziegler, 1991). For both the previous studies and those since 1987, a protective effect of foods rich in β-carotene has been most clearly demonstrated for cancer of the lung.

The problems of interpreting epidemiological, dietary studies have been considered in detail by other reviewers (Mettlin, 1988; Micozzi, 1989; Vogel and McPherson, 1989; Ziegler, 1991). Due to the high correlation of certain nutrients within foods or food groups, it is sometimes difficult to disassociate the effects of one nutrient from another. For example, 2 of the studies described above (Fontham et al., 1988; You et al., 1988) reported a correlation coefficient for intakes of carotene and vitamin C of ≥0.6. The results of some studies were more suggestive of a beneficial effect of vitamin C–rich foods than of β-carotene–rich foods. It would seem reasonable that, depending on which nutrients are more limited in a population's diet, effects might appear stronger for one nutrient than for another, even though both nutrients have beneficial effects. It would be helpful if questionnaires were designed to probe more extensively into the use of foods which are rich in β-carotene but lacking in vitamin C vs those foods with a high content of vitamin C but little β-carotene.

As was pointed out recently by Ziegler (1991) and Willett (1990), constituents of fruits and vegetables other than β-carotene might be important in the prevention of cancer. Some of the questionnaires used in the recent studies inquired about foods items that could help to distinguish between effects of β-carotene and those of other carotenoids, while other questionnaires lacked this discrimination. The importance of other carotenoids and non–carotenoid constituents of fruits and vegetables has not been adequately explored. For example, the fiber component of fruits and vegetables has been implicated as beneficial in prevention of cancers of the colon and rectum (Freudenheim et al., 1990b). In one study in which the food content of nitrate was examined, this food constituent showed a significant inverse relationship with lung cancer (Jain et al., 1990). Nitrate is primarily found in certain vegetables, some of which are also rich sources of β-carotene, so confounding of the effects of these food constituents could also be possible.

C. Evidence for an Optimal Intake of Vitamin A

Most observational studies have examined the relationship of cancer risk to the levels of intake of preformed or provitamin A in the habitual diet, sometimes including supplements as a separate category. Thus, most of our current information is based on usual frequency of intake or, in fewer studies, on a quantitative assessment of the usual dietary intake.

The RDA (NRC, 1989b) for vitamin A (as preformed and provitamin A combined) is 800–1000 Retinol Equivalents (1 RE = 1 μg of retinol). As noted above, the nutritional equivalent of 1 μg of retinol is 6 μg of food carotenoids. In the typical U.S. diet, approximately two-thirds to three-quarters of total vitamin A is preformed, with the remainder supplied by carotenoids with provitamin A activity (NRC, 1989a,b; Ziegler, 1991). The usual intake of β-carotene in the U.S. diet is approximately 1.5 mg/d (Lachance, 1988). A rather similar figure of 2–3 mg/d for Dutch women was reported by de Vet et al. (1991). Lachance (1988) has estimated that, if the current dietary goals of the USDA/HHHS which
advocate increased consumption of carotene-rich foods were met, the intake of β-carotene would rise to nearly 6 mg/d. Generally, differences between the highest and lowest intakes of β-carotene in most cancer studies did not differ by much more than a factor of 2 to 3.

By comparison, only a few intervention trials have tested the ability of supranormal supplements of vitamin A (retinol and/or β-carotene) to affect precancerous conditions or cancer risk [cervical dysplasia (de Vet et al., 1991), skin cancer (Greenberg et al., 1990) or oral leukoplakia (Stich et al., 1988)]. Only in the case of oral leukoplakia, for which supplements equalled 180 mg/week of β-carotene and 100,000 IU/wk of vitamin A, was a positive effect reported. If the rather small differences of 2–4 mg of dietary β-carotene described above are truly protective, then it seems that beneficial effects of supplemental β-carotene should also be seen when results of the ongoing clinical trials are analyzed.

D. EVIDENCE FOR A PROTECTIVE EFFECT OF VITAMIN A

The magnitude of the data supporting a protective effect of dietary vitamin A has been discussed in part in section VI. B. For preformed retinol, the majority of studies did not find evidence of a protective role. In a few studies, a higher intake of retinol did appear to be associated with decreased risk, but for other studies there was evidence suggestive of a positive association with cancer risk. Positive associations have been observed most frequently for cancer of the prostate but also occasionally in studies of colorectal, pancreatic, or breast cancer. In some of the case-control or prospective studies, higher levels of plasma retinol appeared to be related inversely to cancer risk, but the influence of existing or incipient disease on plasma retinol concentration, or analytical differences due to sample storage, could not be ruled out. In all, there is little convincing evidence of a benefit due to higher dietary intake of preformed vitamin A.

Generally, the data are stronger for an inverse association of dietary β-carotene with cancer risk. However, even this association is not highly convincing. For instance, for studies of lung cancer for which the inverse association with β-carotene is most consistent, the relative risk between the groups with the highest and lowest intakes of β-carotene was usually no greater than 2. Confounding by other covariates in carotenoid-rich foods (VI. B.), or by other non-dietary habits such as smoking (see VI. J. below) could significantly influence this small difference in risk. Some of the weakness in demonstrating an association may be due to the quality of the dietary data, which has differed considerably among studies. A number of studies have used food questionnaires of limited scope, have of necessity used proxy interviews, and have assumed standard portion sizes. The limitations in food composition databases, particularly for carotenoids, have been noted above. In retrospective studies, subjects (or surrogates) must recall food intake patterns from the past. In 1 study of a generally well-educated population, the agreement between a baseline dietary report and re-interview 5 years later was only 50 percent for β-carotene and 72 percent for vitamin A supplements (Paganini-Hill et al., 1987), while another found <50 percent agreement for β-carotene after a shorter interval (Bond et al., 1987).

In some of the studies in which serum nutrient levels were measured, a low retinol or β-carotene concentration was a positive risk factor for cancer, while others studies did not find this association. The strength of the inferences that can be drawn from serological studies is highly dependent upon each study's experimental design. In a number of the case-control studies conducted before 1987 and in some of those noted above, blood or plasma had been collected at baseline and stored until clinical cases accrued. Carotenoids are known to be sensitive to oxygen and light, and their instability during long-term storage has been noted before [see ref. (Colditz et al., 1987; Willett, 1990; Ziegler, 1991) for discussion]. There is also evidence that retinol and β-carotene concentrations are lower during illness (Drozdaz et al., 1989; Kanematsu et al., 1989) so that, in prospective studies having a relatively short follow-up, it is possible that results are biased by preclinical disease. For these reasons, the data from
the serological studies conducted to date would seem to be generally less reliable than those from dietary studies.

E. OPTIMAL LEVEL OF CONSUMPTION

It is unknown at what level of consumption of fruits and vegetables there would be no further benefit regarding cancer risk. As noted by Lachance (1988), adoption of the current USDA/HHS dietary goals would increase the average intake of \( \beta \)–carotene by a factor of 2– to 4-fold. A substantial increase in the consumption of fruits and leafy green or yellow–orange vegetables containing provitamin A, balanced calorically by decreased use of fat–rich foods, is achievable and would be expected to increase significantly the consumption of carotenoids and fiber as well as to reduce intake of total fat and saturated fat. Such an overall dietary change would be predicted to reduce the risk not only of cancer but also of cardiovascular disease and diseases related to obesity.

F. TIME COURSE OF BENEFICIAL EFFECTS

It is also unknown how long the benefits of dietary change might persist. However, based on knowledge of epithelial tissues, some speculation is possible. Most epithelial cells are replaced at a regular, relatively rapid, rate. Abnormalities of epithelial morphology and function due to vitamin A deficiency are usually reversed readily after a dietary source of vitamin A is restored. In vitro, the effects of retinol or retinoic acid on cells can often be observed within days or hours. Thus, it is possible that beneficial effects might commence quickly after a change in diet is implemented. On the other hand, studies of induced cancers in laboratory animals have pointed out the need for continuous exposure to vitamin A (or, in most studies, related retinoids) to establish and maintain protection (Moon, 1989; Moon and Mehta, 1990). These data, and knowledge of the latent nature of many cancers, suggest that benefits are likely to accrue slowly from long–term, sustained changes in dietary pattern.

The dynamics of \( \beta \)–carotene are less well known. The types of animal studies which have proved very valuable in understanding vitamin A metabolism are difficult and complicated because most rodents and small mammals absorb carotenoids poorly and store little carotenoid in tissues (Moon, 1989). In humans on a vitamin A–adequate diet, there is considerable storage of vitamin A in the liver as well as \( \beta \)–carotene in adipose tissue and other fatty tissues. Following supplementation with \( \beta \)–carotene, plasma \( \beta \)–carotene concentrations have been observed to increase in less than 2 weeks (Ringer et al., 1991) with a magnitude of change proportional to the \( \beta \)–carotene dose. Based on limited data on tissue carotenoids in humans, there appears to be substantial inter–individual variability in carotenoid storage (Parker, 1989). The relationship of tissue carotenoid level to dietary intake has not been adequately explored.

With respect to cancer, it is not clear at what point during development of disease retinoids or \( \beta \)–carotene would be most likely to exert protective functions. As noted above, there is considerable evidence from carcinogenesis studies in animals that administration of pharmacologic levels of retinoids is effective primarily after tumor initiation (Moon, 1989; Moon and Mehta, 1990). In these studies, protection has depended on continuous retinoid administration (Moon, 1989).

Therefore, from the knowledge at hand, it seems most likely that beneficial effects of dietary modification would begin almost immediately at the cellular level, but that clinical evidence of benefit would be discernable only later for slowly progressing diseases such as cancer. Chronic dietary change would be expected to have greater benefit than intermittent change.
G. POPULATIONS TO WHICH SCIENTIFIC EVIDENCE CAN BE GENERALIZED

The retrospective studies reviewed above have been conducted in many parts of the world with populations whose basic dietary habits differ considerably. There is no evidence that geographical or ethnic differences are important. Within studies that have included men and women, a range of ages, or different ethnic groups, there generally has been consistency. There may be some differences between pre- and postmenopausal women with regard to dietary risk factors for breast cancer, and some of the studies on prostate cancer have raised a question about age-related differences for this type of cancer. Otherwise, it seems most likely that the results of the studies conducted to date may be generalized to the adult population at large.

H. DIETARY INTERACTIONS

There is no substantial evidence that any other components of the diet would interfere directly with a potentially beneficial effect of vitamin A. Experimental studies have shown the efficiency of absorption of \( \beta \)-carotene to be dependent on dietary fat; however, the fat content of nearly all normal human diets is sufficient for optimal \( \beta \)-carotene absorption (Underwood, in VNIS, 1987). The efficiency of carotene absorption varies considerably, and it is possible that carotene bioavailability from some food sources is poor. This has been taken into account as far as possible when the nutritive value of carotenoids has been determined. It is conceivable and even likely that the magnitude of the protective effects that have been seen in cancer studies also depends on the presence or absence of other food components. If nutrients that covary with \( \beta \)-carotene also have protective or deleterious effects, the magnitude of the effects attributed to \( \beta \)-carotene might be an over- or underestimation of its true efficacy.

I. SIGNIFICANT FOOD SOURCES OF VITAMIN A

This subject has been discussed in part in the Introduction and in section II. C. Lachance (1988) has published recent information on the \( \beta \)-carotene content and the usual per capita use of foods which contribute substantial carotenoids. Five food items were found to contribute 85 percent of the \( \beta \)-carotene in the average U.S. diet. These include carrots (256 mg per capita per year), sweet potatoes/yams (122 mg per capita per year), tomatoes (86 mg per capita per year), melons (62 mg per capita per year) and spinach (35 mg per capita per year). Other foods that contribute at least 10 mg per capita per year are lettuce, sweet corn, cabbage/sauerkraut, and broccoli.

Foods rich in preformed vitamin A include beef, calf or chicken livers, kidney, dairy products, and, to a lesser extent, meats and eggs. Vitamin and mineral supplements also generally contain preformed vitamin A in the form of retinyl esters.

J. OTHER TYPES OF DIETARY OR LIFESTYLE INTERACTIONS

Other dietary or health factors, or habits, may significantly influence the effects of dietary retinol or \( \beta \)-carotene on cancer risk, or may have a more powerful effect on cancer risk than either vitamin A or \( \beta \)-carotene. Cigarette smoking, as is now well known, is the strongest, independent risk factor for cancer of the lung. Smoking has also been implicated in cancers of the pancreas and bladder. Numerous studies cited above included smokers. Therefore, the ability of the investigators to adequately match cases and controls and to adjust for smoking habits was critical for the proper interpretation of these data. Differences in the characteristics of smokers and non-smokers have been
noted in various studies. Fehily et al. (1984) found that smokers were lighter and of lower body-mass index and had lower intakes of vitamins, minerals, and dietary fiber than nonsmokers or former smokers. The intake of vitamin A or foods rich in β-carotene has been reported to be highly associated with smoking status, being lower for current smokers than for past- or never-smokers (Ross, 1991; Shibata et al., 1989). Cigarette smoking has also been shown to be inversely related to plasma β-carotene levels in a number of studies (Nierenberg et al., 1989; Palan et al., 1989; Russell-Briefel et al., 1985; Stryker et al., 1988). Smokers had lower plasma β-carotene levels in a dose-dependent manner across a 9-fold range of dietary β-carotene intakes (Nierenberg et al., 1989). Heavy smoking and alcohol intake are also highly correlated (Colditz et al., 1987). These data make clear that tight control of smoking is critical for reliable analysis of dietary studies yet, as discussed by others (Ziegler, 1991), it is not always clear from published work how adjustments for smoking were made and whether these truly controlled for smoking-related behaviors.

Certainly in the case of lung cancer, the relative risk due to cigarette smoking is far greater than the relative risks that have been associated with diets low in vitamin A (Ziegler, 1991). The relationship between diet and lung cancer is modest in comparison to the deleterious effect of cigarette smoking (Willett, 1990).

Alcohol consumption is known to decrease the absorption of many nutrients. Although the studies above did not directly address the interaction of alcohol and vitamin A consumption, alcohol has been shown to be positively related to cancers of the pancreas, esophagus, and other sites. The beneficial effects of diets high in fruits and vegetables, or the use of supplemental β-carotene, would be expected to be antagonized by frequent use of alcohol.

Other health behaviors may influence the outcome of research studies. For example, plasma β-carotene concentration has been reported to be lower in women using oral contraceptives (Palan et al., 1989) while retinol concentrations are increased (Palan et al., 1989; Pilch, 1985). The influence of these behaviors on the outcome of nutritional or epidemiological studies would need to be adequately controlled.

K. SAFETY CONCERNS

There is no apparent cause for concern about reasonable levels of intake such as could be obtained from the diet. As noted above, the USDA/HHS dietary goals call for increased consumption of fruits and vegetables which would, on average, increase β-carotene intake by about 4-fold.

Toxicity has not been evident for β-carotene (NRC, 1989b), even at high doses used in clinical supplementation trials (de Vet et al., 1991; Greenberg et al., 1990; Stich et al., 1988) and in patients treated for years for photosensitive skin disorders (Matthews-Roth, 1989). In the case of high supplemental doses such as those used in the clinical trial of oral leukoplakia of Stich et al. (Stich et al., 1988) or the skin cancer trial of Greenberg et al. (1990), the accumulation of β-carotene in plasma and skin may cause yellowing. Though cosmetically unacceptable to some, this condition is not associated with known toxicity.

There is good reason for concern, however, about toxicity due to chronic overconsumption of preformed vitamin A. Hypervitaminosis A has been reported following self-medication with vitamin A (Smith and Goodman, 1976) and in explorers subsisting on liver and other tissues with a very high vitamin A content (see Lippman and Meyskens, 1988). Symptoms of acute toxicity (nausea, vomiting, or headache) have been reported in a few studies when normal children in populations with low vitamin A status have been supplemented with vitamin A in single doses of 30-60 mg (Florentino et al., 1990; NRC, 1989b).
Toxicity has also been observed in clinical situations in which pharmacologic doses of retinol or retinoids have been used for chemoprevention or chemotherapy (Yob and Pochi, 1987). Side effects and toxic manifestations have involved a number of organ systems including the skin, eyes, liver, joints, bones, and muscles (Yob and Pochi, 1987). For example, toxicity was associated with the highest doses of 13-cis-retinoic acid (100 mg/m² body surface area/d) in a recent prevention trial in patients with previous cancers of the head and neck (Hong et al., 1990). A recent study of adults with advanced acute promyelocytic leukemia who were treated with a high dose of all-trans-retinoic acid (45 mg/m² body surface area/d) showed positive therapeutic effects in some patients, but adverse effects of toxicity were also frequently noted (Castaing et al., 1990). On the other hand, some investigators have reported that administration of high doses of vitamin A for chemotherapy has not been associated with unacceptable side effects. High-dose retinol (approximately 30 times the RDA) has been used chronically to treat children with myelogenous leukemia without apparent, serious toxicity (Liu et al., 1988). Similarly, in a clinical trial of compliance and safety of high dose vitamin A as adjuvant therapy in lung cancer, nearly all patients tolerated doses of ~100 mg/d for over a year without signs of serious liver damage (Pastorino et al., 1991). However, these studies involved careful pre-screening to exclude patients with pre-existing liver disease and, even then, elevations of liver enzymes and of plasma triglycerides were observed during treatment (Pastorino et al., 1991).

Concern is especially great for women of child-bearing age because of the well-known teratogenic effects of high levels of retinol and some of its synthetic analogues (Hall, 1984; NRC, 1989b; Yob and Pochi, 1987). The level of intake causing birth defects, or symptoms of acute toxicity, are well outside those associated with normal dietary practices (NRC, 1989b). Nonetheless, any health message concerning the benefits of vitamin A must be made with great caution because vitamin A supplements almost always contain preformed retinol rather than β-carotene, and the potential for their over-eager use exists. Currently, approximately one-third of the U.S. adult population consumes vitamin supplements regularly, including vitamin A in doses often meeting or exceeding the RDA (NRC, 1989b).

L. DIFFERENCES IN EFFICACY AMONG SOURCES OF VITAMIN A

As commented on above, the bioavailability of carotenoids may vary quantitatively with different food sources. There is evidence that β-carotene supplied in oily solutions is more efficiently converted to retinol, and therefore is of higher potency, than β-carotene in vegetables (NRC, 1989b). Brubacher and Weiser (1985) have estimated that when carotene intake equals 1.5–4.0 mg/d (a normal range), 1 µg of retinol is obtained from 3.33 µg of β-carotene in oily solution or 6 µg of β-carotene in vegetables. Although these data were obtained from animal studies, it seems reasonable that they should also apply to humans. The absorption of retinol or β-carotene, or the conversion of β-carotene to retinol in the intestine, does not appear to vary remarkably between natural foods vs fortified foods such as milk or margarine. There are, however, few experimental studies in this area.

M. CRITICAL GAPS IN KNOWLEDGE

Progress in understanding the absorption, transport, and tissue metabolism of carotenoids has been slow because the small animal species used effectively as models for studies of retinol metabolism do not absorb carotenoids efficiently. It is known that increasing the consumption of β-carotene or other carotenoids leads to a rapid increase in plasma carotenoid concentrations in humans, but little is known of the cellular consequences of such change. It is possible that the organs which take up carotenoids from plasma are able to convert it to retinol, much as occurs in the intestine, or to retinoic acid which could influence differentiation through nuclear receptors and regulation of gene expression. Although the regulation of intracellular binding–proteins and receptors for retinol and retinoic acid
have become an active area of research, very little is known of whether β-carotene, in quantities found in the diet or in supplemental amounts, affects these receptors in any way. A critical area of understanding that is now missing is whether or not β-carotene acts through retinol in the cell, whether the main action of β-carotene is as an antioxidant, or whether it acts primarily through some other process.

The mechanism of transport of retinol and β-carotene into cells is not well understood and constitutes another critical gap in knowledge. It is presently unknown whether the transport protein for retinol (RBP) or the lipoprotein that carries carotenoids (LDL) serve to deliver these molecules to cells in a specific, regulated manner. As vitamin A or β-carotene intake is increased, at what point do these physiological mechanism become limited or altered?

It is known that the dietary habits of tobacco smokers differ from non-smokers, but further detailed information would be helpful both in interpreting epidemiological studies and providing the most acceptable dietary advice for those who continue to smoke. There is also little information on the extent to which smoking alters the requirement for, or metabolism of, β-carotene, other anti-oxidant vitamins, or vitamin A. Studies of absorption, turnover, and tissue metabolism would help to fill this gap in knowledge.

While epidemiological studies of the cohort or case–control design have inherent limitations, they have been and are likely to continue to be important means to identify the potential factors in the human diet that protect, or do not protect, against the development of cancer. Studies of this type can be even more informative in the future if use is made of improvements in the food composition/nutrient database and if there are improvements in the questionnaire/interview methods regarding foods consumption and personal habits such as smoking. For lung cancer, the evidence supporting a protective role of β-carotene is quite strong at the present time. It does not seem overly optimistic to suggest that the addition of a few large, carefully controlled studies could, if consistent, provide a body of evidence that, collectively, would be compelling. Not withstanding interest in the outcome of clinical trials of β-carotene supplementation, there is still a need to evaluate the consequences of normal dietary patterns on disease incidence.

There is also a need to evaluate some of the non-vitamin A constituents of fruits and vegetables (e.g., non-provitamin A carotenoids, indoles, and nitrate) to learn whether these are also protective, or whether the protective effects of dietary vitamin A are exerted by themselves or in combination with other food components.

Regarding cancer prevention, most studies to date have had to rely on cancer incidence or mortality as end points. As the processes leading to clinical disease become better understood, it should be possible to use markers of preclinical disease in place of the later end points. The potential for development of biomarkers for cancer chemoprevention studies was recently reviewed (Lippman and Meyskens, 1988) and appears promising. In principle, such markers should also be useful in dietary studies. Occasionally, epidemiological studies have suggested a positive association of high vitamin A intake with increased risk of certain cancers, such as prostate cancer as discussed above. The significance of these observations is unknown but might indicate covariance of vitamin A intake with other risk factors.

N. SUMMARY

The data relating dietary vitamin A and cancer are inconclusive. The strongest evidence supports a protective role of fruits and vegetables in reducing the rates of cancer of various sites, particularly cancers of the lungs, colon/rectum, and breast. For cancer of the lung, it seems most likely that this
Protective effect is exerted at least in part through the β-carotene contents of these foods. In several studies, the risk of lung cancer was about 2-fold lower in individuals in the highest category of β-carotene intake compared to those in the lowest category of intake. However, the increased risk of lung cancer due to smoking is far stronger than the potentially lower risk associated with diet. Thus, smoking cessation will have a far greater impact on lung cancer rates than can be expected from dietary modification.

For cancers of the colon/rectum and breast, it is possible that the provitamin A component of fruits and vegetables is also protective, but it seems most likely that these foods protect by providing some combination of dietary fiber, provitamin A, vitamin C, reduced fat, or other food constituents.

Based on current knowledge, the dietary goals stated in the Surgeon General's Report and by the USDA/HHS appear to provide excellent directions to a more healthful diet.
V. BIBLIOGRAPHY


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


Mori, S. 1922. The changes in the para-ocular glands which follow the administration of diets low in fat-soluble A; with notes of the effect of the same diets on the salivary glands and the mucosa of the larynx and trachea. John Hopkins Hospital Bulletin 33:357–359.


APPENDIX

CRITERIA FOR INCLUSION OF ARTICLES IN APPENDIX TABLES

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.
## APPENDIX TABLE. VITAMIN A, CAROTENOIDS, AND CANCER (Observational Studies x Site)

### I. Lung Cancer

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<thead>
<tr>
<th>Study</th>
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<tr>
<td>Bond et al., 1987</td>
<td>Nested case-control Texas</td>
<td>308 cases of lung cancer who had died between 1940–80; 308 control subjects living; 308 control subjects deceased (dead ≤ 5 yr after matched case). There were 28 overlapping controls for a total of 556. Controls were individually matched with cases for yr of birth (± 5 yr), race, and yr of hire. This cohort came from a larger pool of employees of a chemical plant.</td>
<td>Telephone interviews with subjects or &quot;next of kin&quot; to collect data about occupational history, tobacco use, residential history, and dietary habits; 29-item food-frequency questionnaire. Items chosen for high vitamin A content Reference period was 3–5 yr prior to development of symptoms in cases and 3–5 yr prior to matched case's death for living controls. No reference period described for decedent group. Interviews were completed in 1984. Analyses were adjusted for cigarette smoking, education, and use of vitamin supplements.</td>
<td>Inverse relationship between vitamin A intake and risk of lung cancer. The association was apparent in comparisons with both controls but significant only in &quot;living&quot; control group. There was a stronger effect for subjects with higher carotenoid index indicating a protective effect of vitamin A from plant sources. A negative association between carrots and lung risk in comparison to living control; a positive association in same comparison between cases and decedent subjects. Cases were reported to have used vitamin supplements more often than either control groups.</td>
<td>Results are presumptive due to lack of biochemistry, extremely long retrospective period (as much as 9 yr in cases), and reliance on surrogate sources (62.3% and 65.1% by spouses or 25.6% and 23.6% by child in cases and decedent controls respectively).</td>
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<tr>
<td>Connett et al., 1989</td>
<td>Nested case-control Minnesota</td>
<td>66 cases of lung cancer out of a total 156 cases of cancer deaths; 311 controls matched for age, smoking status, randomization group, date of randomization, and clinical center. Subjects from a pool of 12,866 subjects involved in an intervention trial (MRFIT) for heart disease</td>
<td>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 -60°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 were by HPLC.</td>
<td>Total carotenoids and β-carotene were significantly lower in lung cancer cases than in their matched controls. There were no differences between total cancer cases and controls. There was significantly reduced risk associated with increased total carotenoid levels; a similar trend (p&gt;0.07) for β-carotene. There was a nonsignificant trend towards lower intake of β-carotene. 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 blood sample storage time on outcomes. Reliability of a single 24-hr dietary recall was questionable. 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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### APPENDIX TABLE. VITAMIN A, CAROTENÖIDS, AND CANCER (Observational Studies x Site)

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<tr>
<td>Dartges et al., 1990</td>
<td>Case–control southwest France</td>
<td>143 cases of epidermoid lung cancer; all histologically confirmed and consecutively admitted 212 hospitalized controls were admitted to the same hospitals with diagnosis other than cancer; matched for sex, age (± 5 yr), residential area, occupation, smoking habits, and alcohol intake.</td>
<td>All subjects were given a structured interview; each case and matched controls were interviewed by same person. Food–frequency questionnaire consisted of &quot;items likely to be part of the normal diet in southwest France.&quot; Subjects asked about frequency of consumption during typical wk. Reference period was the 6-mo period prior to the interview for all subjects.</td>
<td>Consumption of preformed vitamin A and β-carotene was significantly and independently associated with epidermoid lung cancer. The effect did not change with the inclusion of potential confounders, e.g., smoking, into the model.</td>
<td>Estimated intake of vitamin A; no portion sizes used on food–frequency questionnaire; reference period relative to hospitalization rather than onset of disease; unknown duration or stage of disease in cases No community based–controls No supplementation data</td>
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<tr>
<td>Fontham et al., 1988</td>
<td>Case–control Louisiana</td>
<td>1253 cases of lung cancer 1274 controls matched for race, sex, and age (± 5 yr) were all admitted to the same hospitals as cases. There were significantly more non-smokers in the control group (31.5% vs 4.3%).</td>
<td>All subjects were given a questionnaire containing diet and tobacco history, occupational, residential, medical and family health histories. A 59-item food–frequency questionnaire was given with the reference period being before appearance of symptoms. Surrogates were interviewed in both cases (26.7%) and controls (11.5%) were subjects were unable to respond.</td>
<td>An inverse association was found between vitamin C intake and specific types of LC (squamous and small cell). A similar though not as strong effect was found for vitamin A (carotene). There was a significant inverse relationship between retinol intake and adenocarcinoma in blacks.</td>
<td>Time from diagnosis for either groups not given No community–based control group Since comparisons were based on tertiles of intakes for control group, there may have been an underestimation of intake and associated risk due to low intakes by hospital controls. Portion sizes were not estimated by subjects; extrapolated from data on &quot;typical serving&quot;. No comparable reference period for controls No data on supplement use No descriptive data reported for nutrient intake No comparisons to intake standards, e.g., RDA No biochemistry</td>
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### APPENDIX TABLE: VITAMIN A, CAROTENOIDS, AND CANCER (Observational Studies x Site)

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<tr>
<td>Jain et al., 1990</td>
<td>Case–control Toronto, Canada</td>
<td>839 cases of lung cancer; matched pairs of &amp; 772 population-based controls; sex–matched to case pairs Also matched for age (≥ 4 yr) and borough of residence &lt;33% refusal by eligible controls Initial contact of subjects was by mail.</td>
<td>All subjects interviewed to gain data about: SES factors, lifetime residences, occupational history, and detailed smoking history. 81-item food–frequency questionnaire that emphasized vitamin and cholesterol intake Subjects were asked to approximate portion sizes using a reference food models. Data were collected on vitamin and other nutritional supplements. Reference period was 1 yr prior to interview for all subjects. Proxy interviews (primarily spouses) were used for 34% of cases. Time between interview and diagnosis in cases was not reported.</td>
<td>Significantly reduced risk associated with increased intake of vegetables No association between risk and total vitamin A, retinol, vitamin C, or fruit There was an irregular nonsignificant decrease in risk associated with β-carotene. In the small number of supplement users, there was a significant inverse relationship between vitamin A and risk. The form or amount of vitamin A was not available.</td>
<td>Large portion of cases used proxy interviews; 52% of cases interviews were by spouses. Unknown time period between diagnosis and interview in cases could have resulted in long retrospective reference period. Most of the cases (92.5%) were smokers as opposed to 61% of controls.</td>
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<td>Koo et al., 1988</td>
<td>Case–control Hong Kong</td>
<td>88 cases of lung cancer 137 district–matched controls All subjects were &amp; with no known history of smoking.</td>
<td>All subjects were interviewed for demographic data including household number and/or a food–frequency questionnaire. Cases asked about intake 1 yr prior to diagnosis. Controls asked about current intake and were interviewed within 6 wk of matched cases.</td>
<td>Significantly increased risk associated with low intakes of fresh fruit and fish. Protective effect of high consumption of leafy green vegetables, carrots, tofu, fresh fruit, and fresh fish in cases of adenocarcinoma and large cell cancer. Fresh fruits were found to offer protection against squamous cell tumors.</td>
<td>Data analyzed by foods, no analysis for specific nutrients, no biochemistry Retrospective data, large difference in reference intervals: cases, 1 yr prior to diagnosis, controls, current diet Conclusions regarding potential protective effects of vitamin C, retinol, and calcium are presumptive.</td>
</tr>
<tr>
<td>Kune et al., 1989</td>
<td>Case–control Melbourne, Australia</td>
<td>64 cases of lung cancer, consecutively admitted, histologically confirmed 63 hospital controls All subjects were &amp; and not matched for age or any other variables.</td>
<td>All subjects given an interview that included a food–frequency questionnaire of unspecified length. Reference period was not reported. Blood samples were drawn from cases within a few days of diagnosis and before any treatment was initiated. Samples were drawn from controls after admission and before surgery. Fasting status and time between sampling and analysis were not mentioned.</td>
<td>Mean serum levels of β-carotene and vitamin A were significantly lower in cases than controls No difference for serum zinc This effect held after adjustment for age and smoking history. There were no significant associations between dietary vitamin A or dietary β-carotene and serum levels of these nutrients.</td>
<td>No community–based controls, no supplement data reported Cases had very low levels of retinol (30.6 μg/dl) as compared to controls. No diet data reported The authors contend that these results are indicative of a nutritional or metabolic response to the disease rather than a reflection of a role for vitamin A vitamin levels as risk factors. It is difficult to determine whether this is a state or trait phenomena under the conditions reported.</td>
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<td>LeGardeur et al., 1990</td>
<td>Case–control</td>
<td>59 cases of LC 59 hospital controls (HC) selected in a next–patient–encountered procedure 31 community controls (CC) subjects were matched for age (≥ 5 yr), race, county.</td>
<td>LC and HC subjects given a structured interview to obtain data about smoking history and dietary intake (method not described, no reference period given). 20 ml non–fasting venipuncture samples were collected from all subjects. Measures included: serum ascorbate, retinol and carotenoids, vitamin E and cholesterol. Assays were done within a month of collection. CC group was not interviewed only blood was collected.</td>
<td>Mean serum levels of carotenoids, vitamin E, and total cholesterol for LC cases were significantly lower than HC. Retinol levels were lower in cases but not significantly (p&lt;0.07). 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, no questionnaire data for CC group were reported. The CC group was compared to HC group to test for appropriateness of HC as controls for LC group. Test 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., 29% 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 Retinol binding protein was significantly associated with all variables except vitamin C; this could reflect a general malnutrition or a metabolic defect. Insufficient data to make appropriate interpretation. Given the inappropriateness of the controls and poor matching, the use of a paired T–test must be questioned.</td>
</tr>
<tr>
<td>Le Marchand et al., 1989</td>
<td>Case–control</td>
<td>432 cases of LC 260                      102</td>
<td>All subjects given a structured interview to gather data on smoking and alcohol consumption history. Interviews were done at home with the subject or surrogate (29% for cases, 7% for controls). 130–item food–frequency questionnaire was given to all subjects. The reference period was a usual wk, mo, or yr before onset of symptoms for the patients and a corresponding time period for the controls.</td>
<td>Total vitamin A (food and supplements) was inversely associated with risk; not significantly with §. A dose–dependent negative association was found between dietary β–carotene and LC. All vegetables, dark green vegetables, cruciferous vegetables, and tomatoes showed stronger association than β–carotene. An inverse association between total vitamin C (from food and supplements) in § only There was an apparent interaction between sex and race for vitamin C.</td>
<td>Did not control for ethnic differences in intake No biochemistry The authors concluded that the effects associated with vitamin C (resulting from interaction between sex and race) were aberrations that could not be explained by any known biological mechanism and therefore did not explore the vitamin C question further.</td>
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### II. Head and Neck Cancers

<table>
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<tr>
<th>Study</th>
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<tr>
<td>de Vries and Snow, 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 one 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 demographic, 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 16 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 before analysis. Serum vitamins A and E were measured fluorometrically. Serum zinc and copper were measured by atomic absorption spectrophotometry.</td>
<td>Mean levels of vitamin A were lower in cases than either control group. β-carotene levels were also lower in cases than controls (p&gt;0.05). Serum RBP levels were also significantly lower in cases than either control group. There was a significant association between RBP and vitamin A in the cases but not in the controls. Zinc levels were lower and copper levels higher in cases compared to controls. Serum zinc was positively associated with RBP and vitamin A in cases but not controls. There was no difference in levels of vitamin E in any of the group comparisons.</td>
<td>Inappropriate control groups  No diet data or report on supplement use  No matching of groups for age, sex, smoking, occupational exposure, residence, SES  The conclusions about vitamin A or β-carotene are presumptive in the face of a lack of diet history or intake data.</td>
</tr>
<tr>
<td>Franco et al., 1989</td>
<td>Case-control Brazil</td>
<td>232 cases of oral cancer 464 hospital non-cancer controls 2/case matched for sex, age (± 5 yr), and trimester of hospital admission. Neoplastic disease and mental-disordered patients were excluded.</td>
<td>All subjects given a 40 to 60 min structured interview by blinded interviewers. Information included: SES, demographics, general health, environmental and occupational exposure history, tobacco and alcohol use, 20-item food-frequency questionnaire, and oral hygiene habits. No proxy interviews  No reference period reported</td>
<td>Significantly reduced risk associated with smoking and alcohol (the strongest risk factors irrespective of site) adjusted intakes of citrus fruits Without adjustment, significantly reduced risk associated with increased consumption of carotenoid rich foods (e.g., carrots, pumpkins, and papaya) and citrus fruits No protection noted for green vegetables in general</td>
<td>No population-based controls, no biochemistry Retrospective diet data based on limited 20-item questionnaire  No data regarding vitamin C specifically  Conclusions regarding vitamin A or β-carotene presumptive without biochemistry or more extensive comprehensive diet analysis</td>
</tr>
</tbody>
</table>
## APPENDIX TABLE. VITAMIN A, CAROTENOIDs, AND CANCER (Observational Studies × Site)

<table>
<thead>
<tr>
<th>Study</th>
<th>Type/location</th>
<th>Subject # &amp; Description</th>
<th>Methods</th>
<th>Results</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Graham et al., 1990</td>
<td>Case-control</td>
<td>New York 178 (136 m, 42 f) cases of esophageal cancer drawn from a total pool of 743 cases identified during the period of 1975-1986 from 3 counties of western New York State. 178 community-based controls matched for age, sex, race (all white), and neighborhood of residence</td>
<td>Subjects given a structured interview consisting of food-frequency questionnaire covering the previous yr for controls and for cases 1 yr prior to the onset of symptoms. Photographs were used to estimate portion size. Additional information included smoking and alcohol use, occupational and health histories, seasonality of intake, preparation, and food storage.</td>
<td>No risk was observed for vitamin A derived from vegetables or for carotenes alone. There was an observation of a nonsignificant decrease in risk associated with increased intakes of vitamin A from vegetables. In a separate analysis after adjustments for sex, age, education, smoking and alcohol at intakes 1 standard deviation above the range of exposure to several vegetables (lettuce, other greens, tomatoes) were associated with decreased risk. There was a significant increase in risk associated with increased intake of vitamin A from meat and dairy foods and from retinol per se.</td>
<td>The sample represented a small portion (24 percent) of the larger pool of 743 cases identified. The authors discussed the potential for bias in the subjects selected.</td>
</tr>
<tr>
<td>Li et al., 1989</td>
<td>Case-control</td>
<td>Linxian, China 1244 cases of cancer of the esophagus or gastric cardia ages 35-65 yr 1314 controls age- and sex-matched from same geographical area</td>
<td>All subjects given structured interview. Data collected included: demographics, occupation, smoking, diet history by 72-item food-frequency questionnaire, food preparation and storage methods, beverage consumption, anthropomtries, and family and personal health history. Questions were referenced to 2 time periods, the late 1850s and the late 1970s.</td>
<td>All subjects consumed a diet low in fruits and vegetables. No association with risk. Low water and high wheat intakes were associated with increased risk.</td>
<td>Not designed to address specific vitamin A relationship with cancer Not enough variability in intake to assess risk relationship Strong genetic and/or geographical component to risk in this population</td>
</tr>
<tr>
<td>Study</td>
<td>Type/location</td>
<td>Subject # &amp; Description</td>
<td>Methods</td>
<td>Results</td>
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<tr>
<td>McLaughlin et al., 1988</td>
<td>Case-control</td>
<td>871 cases of oral and</td>
<td>All subjects (or next of kin in those cases who were too ill) were given</td>
<td>When derived from fruit there was a significant protective effect of vitamin C, vitamin A, and fiber.</td>
<td>No biochemistry, reliance on 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.</td>
</tr>
<tr>
<td></td>
<td>Four regions:</td>
<td>pharyngeal cancer</td>
<td>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 effect outcomes.</td>
<td>There was an insignificant I in risk associated with intakes of preformed vitamin A (retinol). Intake of dark yellow and cruciferous vegetables was related to I risk in females but not males. Vitamin C was associated with decreased OR (odds ratios) and risk of oral cancer in females and males. Protective effects were seen for fruit consumption. Highest quartile had 1/2 the risk of lowest. No association with calories, methods of food preservation, or cooking. This effect was not apparent for other vegetable sources of these nutrients. No effect for other vitamins or nutrients.</td>
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<tr>
<td></td>
<td>New Jersey,</td>
<td>979 population-based</td>
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<tr>
<td></td>
<td>Atlanta,</td>
<td>controls matched for</td>
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<tr>
<td></td>
<td>Los Angeles,</td>
<td>race (all white), age,</td>
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<td></td>
<td>Santa Clara,</td>
<td>and sex</td>
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<td>San Mateo in</td>
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<tr>
<td></td>
<td>California</td>
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<tr>
<td>Brown et al., 1988</td>
<td>Case-control</td>
<td>207 cases of esophageal</td>
<td>Study I: a hospital-based case control; all patients were interviewed</td>
<td>No association between β-carotene intake and risk. High intakes of retinol were associated with higher risk. After adjustments for smoking and alcohol consumption (the leading risk factors in both studies), significantly increased risks of EC were associated with low intake of fruits, particularly citrus fruits and juices and high intakes of liver. Low vitamin C and fiber intakes were associated with increased risk.</td>
<td>No population-based control group or biochemistry. No portion size estimates. Reliance on retrospective diet data and the use of proxy data in the mortality study. Possibly inappropriate controls in both phases may have lead to conservative estimates of effects. Duration and type of disease in controls may have affected dietary outcomes. Dietary effects may have been secondary to alcohol and tobacco use and or related diseases.</td>
</tr>
<tr>
<td></td>
<td>South Carolina</td>
<td>cancer (EC)</td>
<td>about alcohol, tobacco, diet (65-item food-frequency questionnaire), medical and dental history, occupation, family health history, and demographics. Study II: next-of-kin (usually a spouse or close relative) of the cancer and control subjects was interviewed at home.</td>
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<td>74 hospitalized cases</td>
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<td>and 133 deaths from</td>
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<td>EC during 1977–81. 422</td>
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<td>controls: 157 hospitalized</td>
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<td>no-cancer deaths. The control group for mortality study was matched for race, age, area, and year of death.</td>
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<tr>
<td>Stich et al., 1988</td>
<td>Intervention</td>
<td>130 betel quid chewers</td>
<td>Challenges given in capsules 2x/wk. Total amounts/wk: Group I, 180 mg β-carotene, group II, 180 mg β-carotene + 100,000 IU retinol. The location, size, and appearance of leukoplakia and % of micronucleated cells were evaluated at baseline and 3 and 6 mo. All subjects continued chewing the tobacco mixture throughout the trial.</td>
<td>At 3 mo there was no difference in leukoplakia regression between groups. At 6 mo there was a significant difference in remission and appearance of new leukoplakias between the 2 active groups and the placebo. There was a significant reduction in frequency of occurrence of micronucleated exfoliated cells in both β-carotene groups as compared to controls.</td>
<td>Duration of chewing habit not noted. No dietary controls, no dietary history, no health history.</td>
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<tr>
<td></td>
<td>India</td>
<td>with oral leukoplakia</td>
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<td></td>
<td>divided into 3 groups:</td>
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<td>placebo (n=35), β-</td>
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<td>carotene + vitamin A</td>
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<td>(n=60)</td>
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<tr>
<td>Study</td>
<td>Type/location</td>
<td>Subject # &amp; Description</td>
<td>Methods</td>
<td>Results</td>
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<tr>
<td>Teyns et al., 1987a</td>
<td>Case-control</td>
<td>743 cases (704♂ and</td>
<td>All subjects interviewed about usual food intake with 40-item food-</td>
<td>Higher intakes of several vitamins (retinol, β-carotene, niacin)</td>
<td>No biochemistry</td>
</tr>
<tr>
<td></td>
<td>France</td>
<td>39♀) of esophageal</td>
<td>frequency questionnaire. Portion sizes were estimated. Risk analysis</td>
<td>associated with significantly decreased risk. Higher intakes of</td>
<td>No control for time between diagnosis and study</td>
</tr>
<tr>
<td></td>
<td></td>
<td>cancer</td>
<td>was done first for heavy vs light consumers of individual nutrients,</td>
<td>vitamin C associated with decreased risk. Significant association</td>
<td>No reference period</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1975 controls (822♂,</td>
<td>in a post hoc analysis at 4 levels of consumption, adjustments</td>
<td>between vitamin E intake and relative risk. Cases consumed</td>
<td>No documentation of medications or other treatment</td>
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<tr>
<td></td>
<td></td>
<td>1053♀) from the same</td>
<td>were made for age and 2 levels of alcohol and tobacco consumption,</td>
<td>fewer proteins of animal origin and more proteins of vegetable origin</td>
<td>No data on medical or family health history</td>
</tr>
<tr>
<td></td>
<td></td>
<td>geographical region</td>
<td>and residence (rural vs urban).</td>
<td>and had a higher intake of sugars and starches of vegetable origin.</td>
<td>Hard to determine environmental from genetic effects</td>
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<td>Cases had a lower P.S ratio; oils associated with decreased risk,</td>
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<td>butter associated with increased risk.</td>
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</tbody>
</table>
### APPENDIX TABLE. VITAMIN A, CAROTENOIDS, AND CANCER (Observational Studies x Site)

#### III. Digestive Cancers

<table>
<thead>
<tr>
<th>Study</th>
<th>Type/location</th>
<th>Subject # &amp; Description</th>
<th>Methods</th>
<th>Results</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Charpiot et al., 1989</td>
<td>Case-control France</td>
<td>208 subjects: 70 cases with digestive cancer (DC) 34 patients w/ colonic polyps 78 healthy controls. Cases diagnosed &gt;2 mo prior to study were excluded</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. There were no differences between site and levels of parameters studied.</td>
<td>No dietary intake data or supplement use reported. Aside from a statement about a lack of &quot;denutrition&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>
</tr>
<tr>
<td>Coggon et al., 1989</td>
<td>Case-control England</td>
<td>95 cases (73 F and 22 M) of newly diagnosed stomach cancer 190 age– (± 2 yr) and sex–matched controls</td>
<td>All subjects mailed a questionnaire about food storage and dietary habits including food–frequency questions (consumption of salted and smoked foods, fresh and frozen fruit, and salad vegetables). Follow-up at–home interview to collect or complete forms with interviewer when necessary</td>
<td>Significant inverse relationship between intake of salad vegetables and fruit and risk. Lettuce and tomatoes were most frequently consumed vegetables. High salt intake associated with increased risk.</td>
<td>Global measure of nutrient intake; conclusions about vitamin A or β-carotene would be presumptive. No data on supplement use, alcohol consumption or smoking or health history. Reference period was before onset of symptoms for cases; variable time frame between interview and reference period in cases. No reference period noted for controls.</td>
</tr>
<tr>
<td>Kono et al., 1988</td>
<td>Case-control Japan</td>
<td>139 cases of newly diagnosed gastric cancer (GC) 2574 hospital–based controls (HC) 278 randomly selected community controls (CC) Subjects matched for age and sex</td>
<td>GC and HC subjects interviewed in hospital, CC at home. Data collected included occupational, smoking, and dietary histories. Reference period for all subjects was the year preceding the interview. GC and HC subjects were interviewed before diagnosis.</td>
<td>In comparison with both control groups, there was an inverse relationship between intake of fruits and GC. Also decreased risk associated with increased intake of green tea (&gt;10 cups/d)</td>
<td>No controls for SES (except approximate geographic area). No biochemistry. No data on nature of health problems in HC group. Evidence of relationship between nutrients and GC presumptive as study did not evaluate individual nutrients.</td>
</tr>
</tbody>
</table>
### IV. Colorectal Cancer

<table>
<thead>
<tr>
<th>Study</th>
<th>Type/location</th>
<th>Subject # &amp; Description</th>
<th>Methods</th>
<th>Results</th>
<th>Comments</th>
</tr>
</thead>
</table>
| Freudenstein et al., 1990a | Case–control New York       | 422 cases of rectal cancer (277 #, 145 %) 422 sex-, race-, age (≥5 yr)-, neighborhood- matched controls | Subjects given a 2.5–hr interview consisting of food–frequency questionnaire covering the previous yr for controls and for cases a yr prior to the onset of symptoms. Additional information included smoking and alcohol use, occupational and health histories, seasonality of intake, preparation, and food storage. | Decreased risk with increasing intake of carotenoids, vitamin C, and dietary fiber from vegetables  
No association between intake of vitamin E and risk  
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 "only relatively alert, healthy subjects could tolerate the 2.5 hr interview")  
Well-conceived study |
| Graham et al., 1988    | Case–control New York       | 428 cases of colon cancer (CC) 428 controls matched for age, sex, and neighborhood       | All subjects were given a structured 2.5–hr interview similar to that used by Freudenstein et al.  
No reference period was noted for the diet data.  
No surrogates were used. | No significant risks associated with intake of protein, vitamin A from vegetables and fruits, carbohydrates, vitamin C, cruciferous vegetables, calcium, or phosphorous. There was significantly reduced risk associated with high intakes of tomatoes, peppers, carrots, onions, and celery.  
Risk of CC was positively associated with increasing intake of total fats (predominantly animal fat) and total calories. | No reference period for food–frequency questionnaire given  
No data on supplement use |
| La Vecchia et al., 1988 | Case–control Italy          | 339 cases of colon cancer (CC) 236 cases of rectal cancer (RC) 778 hospital controls admitted for acute, non–neoplastic or digestive disorders | All subjects given a questionnaire to obtain data on: SES, smoking, alcohol, coffee and other methylxanthine containing drinks, personal and family health history, and use of selected drugs. 29–item food–frequency questionnaire.  
Reference period was an unspecified period before current hospital admission. Subjects also asked to report changes over previous 10 yr | Risk of both CC and RC was inversely related to intake of green vegetables, tomatoes, melon, and coffee. There was also an inverse relationship between risk and indices of carotenoid and vitamin C intake. Consumption of pasta and rice associated with increased risk of both cancers. | No supplement data, variable reference times between cases and controls, no population–based controls  
Diet database was small (only 29 items)  
Individual nutrient estimation unreliable due to lack of portion size information  
No biochemistry  
No descriptive data or comparisons to normal standards of intake |
<table>
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<tr>
<th>Study</th>
<th>Type/location</th>
<th>Subject # &amp; Description</th>
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<th>Comments</th>
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<tbody>
<tr>
<td>Kune et al., 1987</td>
<td>Case-control Melbourne, Australia</td>
<td>715 cases of colorectal cancer, CRC (392 colon cancer, 323 rectal cancer) all histologically-confirmed new cases 727 age- and sex-matched community controls 159 hospital controls</td>
<td>300-item food-frequency questionnaire used to ascertain usual daily consumption Serving sizes were estimated by subjects. Calculated average weekly amounts adjusted for seasonal variations Reference period was the previous 20 yr. Data included use of vitamin supplements.</td>
<td>There was a dose-dependent inverse relationship between fiber, vitamin C, β-carotene, total vegetables, and cruciferous vegetables. β-carotene was highly correlated with vegetable intake. Dietary retinol had no independent association with risk of CRC. Dietary vitamin C was protective at intakes &gt;230 mg/d. The intake of vitamin supplements was highly protective.</td>
<td>Long retrospective diet period. Supplement data not clearly presented (multivitamins or individual, quantity, or interaction with diet).</td>
</tr>
<tr>
<td>Touyz et al., 1987b</td>
<td>Case-control Belgium</td>
<td>453 cases of colon cancer (CC) 365 cases of rectal cancer (RC) 2851 controls All subjects were from same 2 provinces and adjusted for age and sex. All subjects interviewed about diet using food-frequency questionnaire. Reference period for cases 1 wk period prior to onset of disease, controls current intake. Portion sizes were estimated using food models (pictures). Cases interviewed in hospital, controls at home.</td>
<td></td>
<td>Intake of retinol and vitamin B2 was higher in cases; intakes of β-carotene and vitamin C were lower in cases. Significant positive associations were found for retinol, oligosaccharides; negative associations for fiber, linoleic acid, thiamine, and iron. After adjustment for age, sex, province, and caloric intake, retinol was positively associated with CC and RC; significant negative associations with fiber, thiamine, vitamin B6, iron, and vitamin C (for RC only). Retrospective data collection. Differences in reference period between controls and cases. Group differences by province and sex. No biochemistry, supplementation data, descriptive data on intake, demographics, smoking, alcohol, medical histories, or comparisons to normal standards for intake. Possible bias from place of interview; hospital for cases, home for controls. There was no discussion of the food sources of retinol that might have contributed to its effect. No discussion of relationship of foods and outcomes, i.e., grains as sources of thiamine, fiber, etc.</td>
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<tr>
<td>West et al., 1989</td>
<td>Case-control Utah</td>
<td>231 cases of newly- (within 6 mo) diagnosed colon cancer (CC) 391 controls matched by age (± 5 yr), sex, and county of residence. All subjects were interviewed in their homes. Questionnaire consisted of demographic, health history, current height and weight (2 yr before interview), computed body-mass index, physical activity, and dietary data. 99-item food-frequency questionnaire. Reference period was 2-3 years prior to the interview. Portion sizes were estimated with food models.</td>
<td></td>
<td>Significant protective effects of β-carotene in ♂ and ♀; cruciferous vegetables in ♀; fiber was protective in ♀. No association between CC risk and intake of vitamin C or A (presumably retinol) after adjustment for age, BMI, fiber, and energy intakes.</td>
<td>Unknown relationship between reference period and time of diagnosis in cases No biochemistry, comparisons to intake standards or descriptive statistics No data for SES, alcohol, smoking, or supplement history</td>
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</table>
### APPENDIX TABLE. VITAMIN A, CAROTENOIDs, AND CANCER (Observational Studies x Site)

#### V. Pancreatic Cancer

<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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<tbody>
<tr>
<td>Burney et al., 1989.</td>
<td>Nested case-control Maryland</td>
<td>22 cases of pancreatic cancer (PC) 44 controls matched for age, race, sex, and hr between the blood sampling and last meal</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>
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<tr>
<td>Falk et al., 1988</td>
<td>Case-control Louisiana</td>
<td>363 cases of pancreatic cancer (PC) 1234 hospital-based controls (HC) matched on hospital of admittance, race, sex, and age (≥ 5 yr)</td>
<td>All subjects were given an interview to obtain data on smoking, occupational and residential history, alcohol use, family health history, medical history, leisure time activities, and diet. A 59-item food-frequency questionnaire was used. Indices for vitamins A (total) and C, retinol, and β-carotene were created. The reference period was the time (unspecified) prior to diagnosis or onset of symptoms. &lt;50% of cases were unable to be interviewed, surrogates (next of kin, usually a spouse) were used. 3% of controls were unavailable.</td>
<td>Fruit consumption (fresh and juice) was inversely related with PC. There was a smaller nonsignificant inverse association with vegetable intake. No differences in risk associated with vitamin A, retinol, or carotene intakes. Trend analysis indicated increased risk with vitamin A in both sexes (significantly in φ); there was a 1 trend in risk associated with carotene in φ. After adjustment for fruit intake, a nonsignificant inverse association was found for φ in highest levels of carotene index. Risks associated with consumption of fruits and with an index of vitamin C showed significant decreasing gradients across sexes. Cigarette smoking was a strong risk factor for PC.</td>
<td>Control diet of unknown quality used as reference. No descriptive data reported nor comparisons of diet to reference standard, i.e., RDA. No community-based control group. Unknown time period between time of interview and diagnosis and/or onset of symptoms. Controls diet response reflected recent intake patterns. No data on supplement use. No biochemistry. No testing for the potential interaction between smoking and vitamin C index or fruit consumption.</td>
</tr>
<tr>
<td>Farrow et al., 1990</td>
<td>Case-control Washington (state)</td>
<td>148 married φ cases of PC diagnosed between 1982-1986; 188 controls randomly selected and frequency-matched by age (≥ 5 yr); All were married and φ.</td>
<td>Data were collected from surrogates (wives) in 2 steps. A telephone interview to collect demographic data, medical and occupational history, and use of tobacco, alcohol, coffee, and vitamin supplements Dietary questionnaire was mailed and contained a 135-item food-frequency questionnaire. Reference period was 3 yr prior to diagnosis.</td>
<td>No association between PC risk and intake of vitamin A or total fat, saturated fat, cholesterol, ω-3 fatty acids, or vitamin C. No difference between groups in their use of supplemental multivitamins, vitamin A, or C.</td>
<td>Reliability and validity of data acquisition is questionable. Reliance on retrospective data collected from surrogates. Reference period was 3 yr prior to interview.</td>
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<tr>
<td>Study</td>
<td>Type/location</td>
<td>Subject &amp; Description</td>
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<tr>
<td>Mills et al., 1988</td>
<td>Cohort California</td>
<td>Study population was 34,198 non-Hispanic Seventh-day Adventists &gt;25 yr of age. 40 cases of death from pancreatic cancer (PC) occurring during the follow-up period of 1974-1982</td>
<td>All subjects completed a lifestyle questionnaire, details of which were not supplied.</td>
<td>Current use of meat, poultry, or fish was associated with increasing risk. There was a significant increase in risk associated with increasing consumption of eggs. Intake of vegetarian protein products, legumes, and dried fruits was significantly inversely related to risk. No relationship between risk and intake of other fresh fruit, canned or frozen fruit, fresh citrus fruit, fresh winter fruit, green salads, or cooked green vegetables. These results were age- and sex-adjusted.</td>
<td>Problems include: no comparison group, no data on quality of diet, no details on diet data, no data on individual nutrients, no data on supplement use, no biochemistry, no demographics.</td>
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</table>
**Appendix Table. Vitamin A, Carotenoids, and Cancer (Observational Studies × Site)**

**VI. Liver Cancer**

<table>
<thead>
<tr>
<th>Study &amp; Date</th>
<th>Type/location</th>
<th>Subject # &amp; Description</th>
<th>Methods</th>
<th>Results</th>
<th>Comments</th>
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<tbody>
<tr>
<td>Hanematsu et al., 1989</td>
<td>Cross-sectional Japan</td>
<td>28 patients consecutively admitted for hepatic resection; 21 <em>♂</em> and 6 ♀; 10 cases of human hepatocellular carcinoma (HCC); 19 patients had cirrhosis, 4 had fibrosis, 1 had chronic active hepatitis, and 1 had none of these conditions.</td>
<td>Fasting AM blood samples were 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>Statistically significant difference in levels of retinol between tumor and adjacent &quot;normal&quot; cells. No difference in vitamin E or cellular RBP levels. 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>
</tr>
<tr>
<td>Ostrowski et al., 1987</td>
<td>Case-control Poland</td>
<td>23 cases of cancer deaths (11 <em>♂</em> and 12 ♀): 4 died from lung cancer, 4 breast cancer, 4 gastric cancer, 4 colon cancer, 3 gall bladder cancer, 3 cervical or ovarian cancers, and 1 pancreatic cancer. 19 also had metastatic disease in the liver. Controls were from 34 patients who died from heart or respiratory disease, diabetes mellitus, or diseases of the CNS.</td>
<td>Liver necropsy samples were obtained within 24-hr postmortem and analyzed for retinol.</td>
<td>Median retinol levels were significantly lower in cases than controls. All of the cases had levels considered very low (&lt;35 μg/g tissue). 71% of controls had low levels.</td>
<td>Inappropriate controls, no diet data, no nutritional or health history. Not a study of vitamin A nutrition.</td>
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### APPENDIX TABLE: VITAMIN A, CAROTENOIDs, AND CANCER (Observational Studies x Site)

#### VII. Breast Cancer

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<tr>
<th>Study</th>
<th>Type/location</th>
<th>Subject # &amp; Description</th>
<th>Methods</th>
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<tr>
<td>Basu et al., 1989</td>
<td>Case-control Canada</td>
<td>30 cases w/advanced stage breast cancer (BC) with distal metastases 29 cases with benign breast disease (BBD) 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. Analyses were blinded as to subject category. Vitamin A (retinol), vitamin E, selenium, prealbumin, RBP, and β-carotene were measured.</td>
<td>Levels of all nutrients studied (vitamin A, β-carotene, vitamin E, and selenium) were lower in the BC group than in controls, although not significantly. For retinol, RBP, and prealbumin the magnitude of difference was decreased in comparison of BC to BBD group. In comparison across all groups, only RBP was statistically significant.</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>
</tr>
<tr>
<td>Brisson et al., 1989</td>
<td>Case-control Quebec, Canada</td>
<td>290 cases of newly diagnosed breast cancer 645 age-matched control &amp; without BC enrolled in a longitudinal BC screening program/study</td>
<td>All subjects were interviewed about demographics, menstrual history, height and weight, smoking history, physical activity, drug use history, and diet history. 114-item food-frequency questionnaire. Reference period: the previous year. Food models were used to estimate the size of portions. All subjects had mammograms; for cases the unaffected breast was evaluated, for controls random selection was used. All evaluations were done blindly.</td>
<td>1 carotenoid and fiber intakes were associated with a 1 in high risk features on mammograms. Retinol had no effect on mammograms features. In controls, 1 in energy-adjusted saturated fat intake was associated with 1 high-risk mammographic features.</td>
<td>No comparisons in terms of risk of BC related to intake. Analysis limited to associations between mammogram features and diet. Supplement use not reported. Dependent variables (mammogram features) were derived from subjective evaluation of the observer. The relationship of mammographic features to dietary components and breast cancer risk factors was assessed in controls only. The dietary design of this study is cross-sectional, not case-control. Potential for self-selection bias as all controls were from a pool of volunteers involved in an ongoing BC screening program.</td>
</tr>
<tr>
<td>Ewertz and Gill, 1989</td>
<td>Case-control Denmark</td>
<td>1474 cases of breast cancer diagnosed over period 1983–1984 1322 age-stratified randomly-selected controls</td>
<td>Cases mailed questionnaire 1 yr after diagnosis. Controls matched to cases for date of diagnosis. 21-item food-frequency questionnaire designed to &quot;include 80% of the consumption of fat and β-carotene in the study population.&quot; Food models were used to estimate portion sizes. Subjects also asked about use of supplements, caffeinated beverages, sugar, and artificial sweeteners</td>
<td>There was no association between β-carotene intake and risk of breast cancer. There was a significant trend for increased risk with increased intake of total fat. There were no changes in these findings after adjustment for SES, age at first menarche, natural menopause, parity, and age at first birth. Nonsignificant elevation in risk with the use of all common vitamin supplements. More cases (72%) than controls (67%) used supplements.</td>
<td>Reference period not clearly defined. The rationale stated for delay of a yr after diagnosis was &quot;to avoid asking questions on diet during a period where adjuvant chemotherapy was administered...&quot; This implies that the diet data referred to intake patterns after diagnosis which would not reflect risk, rather response to the disease.</td>
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## APPENDIX TABLE. VITAMIN A, CAROTENOIDs, AND CANCER (Observational Studies x Site)

<table>
<thead>
<tr>
<th>Study</th>
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<tr>
<td>Hislop et al., 1990</td>
<td>Case-control</td>
<td>124 cases of proliferative benign breast disease categorized by 2 criteria for risk</td>
<td>Data collected from all subjects included self-administered 39-item</td>
<td>Proliferative benign breast disease was inversely associated with vitamin A supplementation and frequent green</td>
<td>No biochemistry&lt;br&gt;Reference period not clear with respect to health history&lt;br&gt;No comparisons with breast cancer patients&lt;br&gt;Self-selection bias possible as all participants were enrolled in breast cancer prevention program Vitamin A supplements not characterized</td>
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<td></td>
<td>Vancouver, Canada</td>
<td>controls were selected from a pool of participants in a breast cancer screening program.Subjects matched on year of birth (± 1 yr)</td>
<td>food-frequency questionnaire. Items selected for fat, β-carotene, and</td>
<td>vegetable consumption. Severe changes and borderline carcinoma were significantly associated with fat consumption. There was no relationship between vitamin A or vegetable consumption and high risk groups.</td>
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<tr>
<td>Howe et al., 1990</td>
<td>Case-control</td>
<td>4437 cases of breast cancer (BC) 4341 population controls 1754 hospital controls</td>
<td>Analysis included all studies of diet and BC completed by 1986. Authors made diet data available from each of 12 studies. Where data had not been available, estimates of intake were made using food frequency questionnaires. Data on vitamin C was available from 9 of 12 studies. Pre- and postmenopausal γ were analyzed separately.</td>
<td>Vitamin C had the most consistent statistically significant inverse association with BC risk. There were no significant changes in risk ratios associated with any nutrient in the premenopausal group. Significant negative association with risk for total vitamin A, β-carotene, fiber, and vitamin C in postmenopausal and the combined group (pre- and post-menopausal). Retinol was not associated with risk at any level. Vitamin A effect was due to β-carotene. There was a significant positive association between saturated fat intake and risk in postmenopausal cases.</td>
<td>Questions about the validity of meta-analysis in terms of lack of control of independent variables that could influence outcomes, i.e., socioeconomic status (SES), supplement use, clinical stage, medications, smoking habits, and reliability of dietary data</td>
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<tr>
<td>Katsouyanni et al., 1996</td>
<td>Case-control</td>
<td>120 breast cancer cases 120 hospital controls (patients in orthopedic ward in a different hospital than BC cases)</td>
<td>All subjects interviewed before discharge on first hospital admission. Data collected included: demographics, socioeconomic, reproductive, and medical histories. 120-item food-frequency questionnaire. To assess the impact of individual nutrients on BC, nutrient intakes were calorie adjusted.</td>
<td>Total vitamin A intake was inversely associated with BC risk. Cases consumed less total vitamin A and retinol than controls. There was no difference in adjusted β-carotene intake. There were no differences in actual or calorie-adjusted intakes of vitamin C between cases and controls. Similarly, there was no association between vitamin C and risk of BC.</td>
<td>Inappropriate controls (about 25% had osteoarthritis which is known to affect antioxidant vitamin status)&lt;br&gt;Potential mismatching due to different catchment area of controls&lt;br&gt;No demographics; no biochemistry&lt;br&gt;Diet data was related to the period preceding the onset of the disease which was not controlled nor was it documented.&lt;br&gt;Supplement use not documented&lt;br&gt;Data collected over a 12-mo period; no control for seasonal variations in intakes&lt;br&gt;Portion sizes were estimated from averages in food tables.</td>
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<td>Marubini et al., 1988</td>
<td>Case-control</td>
<td>214 new cases of breast cancer, treatment-naive consecutive admissions; 215 controls consecutively admitted to hospital; exclusions were malignant, hepatic, vascular, or metabolic disease.</td>
<td>Fasted blood drawn d after admission and frozen at −18°C. Levels of retinol, β-carotene, vitamin E, vitamin C, and riboflavin were assessed. Subjects interviewed about demographics and medical history. 69-item food-frequency questionnaire was used to assess dietary habits. Reference period was the previous yr unless diet had changed, in which case subjects were asked about the previous 12 mo. Subjects estimated portion sizes.</td>
<td>Mean blood levels of retinol were significantly higher in case than controls. β-carotene levels were also higher but not significantly. No differences in intakes of either vitamin between groups. No difference in risk or odd ratios trend for risk between groups.</td>
<td>Unclear reference period Number of subjects with dietary changes within yr not documented Blood levels taken after admission to hospital + an overnight fast might not be an accurate reflection of status. No community-based controls No supplement data and no analysis by food group</td>
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<tr>
<td>Potischman et al., 1990</td>
<td>Case-control</td>
<td>83 cases of breast cancer; 113 controls; Subjects classified according to breast biopsy; 79 controls had benign lesions.</td>
<td>Self-administered questionnaire about health history and dietary practices. 38-item food-frequency questionnaire (items chosen to represent 90% of variability of vitamin A intake). Reference period was period immediately prior to admission. Portion sizes estimated from &quot;standard portion sizes.&quot; Fasted blood drawn before biopsies and stored at −80°C until the end of the study.</td>
<td>Cases had significantly lower levels of β-carotene and lycopene than controls. No difference in vitamin A intake, or blood levels of α-carotene and retinol. Adjusted multivariate analysis showed an inverse relationship between risk and levels of β-carotene that was restricted to post-menopausal ♀ (cases were significantly older than controls). No effect of plasma retinol but a significant trend with 1 retinol and 1 β-carotene. No association between dietary vitamin A (total or from vegetable sources) and risk.</td>
<td>Potentially inappropriate controls as there were no differences in known risk factors between groups (indicated that this was a high-risk group). No community-based controls Self-reported diet data did not include supplement use or reference period related to disease onset. No portion sizes reported by subjects</td>
</tr>
<tr>
<td>Rohan et al., 1990</td>
<td>Case-control</td>
<td>383 biopsy-confirmed cases of benign proliferative epithelial disorders (BPED); 192 controls without BPED (confirmed by biopsy); 363 community-based controls</td>
<td>All subjects given a standardized questionnaire at home. Cases and biopsy controls interviewed just after diagnosis (intervals 2.8 and 2.9 mo, respectively). 179-item food-frequency questionnaire. Cases and biopsy controls were asked to record intake prior to diagnosis and disregard any changes made subsequent to diagnosis.</td>
<td>Statistically significant trend towards decreased risk with 1 intake of retinol and β-carotene when cases compared to community controls. There was a similar trend with biopsy controls but not statistically significant. Adjustment for energy intake eliminated the β-carotene trend but not the point estimate.</td>
<td>Portion sizes estimated No analysis by food group No data on supplement use Case group may have been self-selected as they differed from controls in self-examination practices.</td>
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### APPENDIX TABLE. VITAMIN A, CAROTENOIDS, AND CANCER (Observational Studies & Site)

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<tr>
<td>Rohan et al., 1988</td>
<td>Case-control</td>
<td>451 cases of breast cancer histologically confirmed 451 controls matched by residential area (same city) and age (± 1 yr)</td>
<td>All subjects given a standardized interview at home. For cases the average interval between diagnosis and interview was 4.8 mo. Family and personal health history and SES data collected. Diet data collection similar to Rohan et al., 1990. Cases were asked to disregard any dietary changes made since diagnosis.</td>
<td>No change in risk associated with retinol intake. Significantly reduced risk associated with β-carotene intake across the whole sample. No effect associated with energy, protein, or total fat consumption. In premenopausal ♀, risk 1 with 1 retinol intake and 1 with 1 β-carotene intake. In postmenopausal ♀, risk was highest in the second lowest quintile of consumption.</td>
<td>See Rohan et al., 1990. Study subjects were asked to record usual dietary patterns and to disregard reporting of changes in dietary patterns that occurred after diagnosis. Reference period included time before and after diagnosis and possible treatment.</td>
</tr>
<tr>
<td>Toniolo et al., 1989</td>
<td>Case-control</td>
<td>250 cases of breast cancer (free of metastases, except in regional lymph nodes) Controls were 499 ♀ from general population stratified by age (±10 yr) and geographical area.</td>
<td>All subjects interviewed (unblinded) given modified food frequency questionnaire structured by meals. Cases interviewed on average of 7.8 mo after diagnosis and after treatment or surgery. Indigenous foods and recipes were added to the data base. General demographic data was obtained from electoral rolls. Standard portion sizes before cooking were estimated. Interview data included SES data, health, and reproductive history.</td>
<td>Intakes of retinol and β-carotene were slightly higher in cases. No difference in vitamin E or C intake between groups. Reduced risk was associated with decreased intakes of fat especially saturated fat and animal protein.</td>
<td>Not blinded, no biochemistry, long period of time between diagnosis or treatment and study (on average 7.8 mo after diagnosis). Retrospective diet data not necessarily indicative of diets prior to diagnosis Smoking histories not reported</td>
</tr>
<tr>
<td>Van't Veer et al., 1990</td>
<td>Case-control</td>
<td>133 newly diagnosed cases of breast cancer 238 community controls</td>
<td>All subjects were given a home interview about demographics, smoking history, health, and reproductive and hormone history. No interviews during chemotherapy 236-item food–frequency questionnaire Reference period 12 mo prior to diagnosis in cases and 12 mo preceding interview in controls. Portions were estimated by subjects using common household utensils, e.g., spoons, plates, cups.</td>
<td>There was no difference in intake of β-carotene between cases and controls. There was a nonsignificant inverse trend for risk in the age-adjusted comparison of highest to lowest intakes of β-carotene. There was a statistically significant trend for 1 risk associated with 1 intake of cereal (90% of cereals from bread). Cases had a statistically significant lower energy adjusted intake of dietary fiber. There was a nonsignificant inverse trend in risk associated with fiber intake. Non-significant lower risk with 1 intake of β-carotene, fruit, and vegetables.</td>
<td>Small sample size Large nonresponse rate in selection of controls (238 out of a potential pool of 548) may have resulted in bias. Cases and controls not matched on reference period, i.e., time between diagnosis and interview in cases No supplement data and no analysis with other risk factors, e.g., smoking, hormones</td>
</tr>
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### APPENDIX TABLE. VITAMIN A, CAROTENOIDS, AND CANCER (Observational Studies x Site)

#### VIII. Cervical/Ovarian Cancer

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<tr>
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<tbody>
<tr>
<td>Brock et al., 1988</td>
<td>Case-control Australia</td>
<td>117 cases of cervical cancer 190 controls matched for SES, age (± 5 yr) 100 of the interviewed cases agreed to blood sampling, 143 of the controls</td>
<td>All subjects interviewed either at home or at work. Cases interviewed within 6 mo of diagnosis. Questioned on demographics, reproductive, contraceptive, and gynecological factors. 160–item food–frequency questionnaire with an emphasis on vitamins A, C, and folate. Reference period was the previous year. Photographs of food were used to estimate portion sizes. Blood collected after an overnight fast and assessed for β-carotene, retinol, and carotene with HPLC methods.</td>
<td>Cases were not matched on sexual habits, smoking, or use of oral contraceptives. Crude risk estimates showed a significant protective effect from 1 intake of carotene, vitamin C, and folate. After adjustment for known risk factors the protective trends for all except vitamin C (p&lt;0.07) disappeared. When considered together, vitamin C, fruit juices, and plasma β-carotene showed a significant protective effect. Fruits did not show a protective effect. Current supplement use was associated with a &quot;marginally significant&quot; reduction in risk while past use was not. No other comparisons of supplements to intake, blood levels, or other risk factors were made.</td>
<td>No vitamin C biochemistry Blood sampling of cases may have reflected state vs trait phenomena. Intakes of food or amounts or type of supplements used was not reported. Only quartile comparisons were made for intake data.</td>
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<tr>
<td>Cuzick et al., 1990</td>
<td>Case-control London, England</td>
<td>45 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>No significant differences in vitamin A were found between groups. There were no significant trends in risk associated with vitamin A. 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 for smoking, sexual behavior, and use of oral contraceptives were made.</td>
<td>No diet data or reported use of supplements No matching for SES Vitamin A levels not defined (presumably retinol) No discussion of β-carotene</td>
</tr>
<tr>
<td>de Vet et al., 1991</td>
<td>Intervention trial randomized placebo-controlled multiblinded Netherlands</td>
<td>369 ≥ ages 20–65 with untreated cervical dysplasia were the subject pool.</td>
<td>Subjects stratified by age, hospital of diagnosis, and degree of dysplasia (3 categories) 137 received 10 mg β-carotene for 3 mo 141 received placebo At 2 mo, asked about diet habits</td>
<td>The number of patients who had progression was not enough for comparisons. There was no effect of supplement on regression of cervical dysplasia.</td>
<td>No biochemistry Diet data collected in the middle of the trial No baseline diet data nor was there an appropriate reference period Selective use of food–frequency and portion–size estimation (only done for some foods)</td>
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<td>Study</td>
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| Palan et al., 1989    | Cross-sectional        | 18 × with uterine leiomyoma 5 × with uterine cervix cancer 2 × with cancer of endometrium, ovary, breast, and colon and 1 × with cancer of lung, liver, and rectum | Normal, benign and malignant tissues from uteri (hysterectomies), and various other carcinoma sites, i.e., uterine cervix, endometrium, ovary, breast, lung, liver, colon, and rectum were collected from the same group of patients. Adjacent normal tissue was used for comparison to cancer tissue within each subject. Tissues were analyzed for β-carotene content. | β-carotene was significantly lower in fibroid tissue than normal myometrium. The concentrations of β-carotene were lower in all cancer tissues when compared to adjacent ‘normal’ tissue. | Not a nutrition study  
No diet data, no supplement use data  
No nutritional biochemistry  
Results could reflect a consequence of the disease rather and reflection of an active role of β-carotene in the cancers studied.  
Small sample size may be too small to establish statistical significance. |
| Palan et al., 1988    | Case-control           | 32 cases of cervical cancer 72 subjects with abnormal pap smear divided into 2 groups: 37 mild and 35 severe dysplasia 37 controls recruited from a family-planning clinic who were using barrier method of contraception. | Blood samples were collected 2–3 hr after breakfast, frozen, and stored at −80°C for not more than 1 wk prior to analysis for retinol and β-carotene. Samples were collected prior to any treatment. | Mean plasma β-carotene was significantly lower in all dysplasias and cancers when compared to normal group.  
There was significant difference between mild and severe dysplasia and between both dysplasias and cancer with cancer having the lowest levels. There were no differences in plasma retinol levels between the groups. | See comments for Palan et al., 1989 |
| Shu et al., 1989      | Case-control           | 172 cases of epithelial ovarian cancer 172 cases matched for age (± 5 yr) and residence | All subjects interviewed about demographics, reproductive history, personal and family health histories, occupational history, and diet. 63 indigenous item food–frequency questionnaire about normal adult consumption. Subjects were asked to estimate portion sizes. | No effect of dietary vitamin A or β-carotene  
Significant increased risk associated with total and saturated fat | No time frame between interview and diagnosis given; no reference period given for cases.  
No biochemistry, no supplement data, no descriptive statistics or comparisons to known standards of intake  
Groups were not matched for SES, cases were more educated. |
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<tr>
<td>Slattery et al., 1989</td>
<td>Case-control</td>
<td>Utah</td>
<td>85 cases of primary ovarian cancer 492 population-based controls matched for age</td>
<td>After adjustment for age, number of pregnancies, and the body-mass index, there was significantly reduced risk associated with α-carotene intake. No effect of vitamin C Small nonsignificant decrease in risk associated with vitamins A and C and fiber</td>
<td>Used nutrient analysis rather than food groups No supplement data Low response rate in cases</td>
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<tr>
<td>Verreault et al.,</td>
<td>Case-control</td>
<td>Washington (state)</td>
<td>189 cases of cervical cancer 227 controls age-matched and identified by random-digit-dialing methods</td>
<td>High intakes of carotene were associated with unadjusted 1 risk of squamous cell cancers. After adjustment for total energy intake and known risk factors there was a nonsignificant trend for 1 risk. After adjustment for known risk factors, increased intake of dark green or yellow vegetables, and fruit juices were associated with significantly reduced risk. There was no association between retinol intake and risk. Decreased risk associated with high intakes of vitamins C and E</td>
<td>No portion sizes given on food–frequency questionnaire; portion sizes estimated from food composition tables. No biochemistry Use of a long retrospective period; the average delay between interview and reference period was 2.8 yr for cases and 2.7 yr for controls</td>
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### Appendix Table. Vitamin A, Carotenoids, and Cancer (Observational Studies x Site)

#### IX. Prostate Cancer

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<th>Study</th>
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<tr>
<td>Hayes et al., 1988</td>
<td>Case-control, Netherlands</td>
<td>94 cases of clinical prostate cancer, 40 cases of focal prostatic cancer, 130 cases of benign prostatic hyperplasia, 130 hospital controls (admitted for either orthopedic or pulmonary surgery) Dutch literacy was required; subjects did not have to be natives. Cases were either new admissions or were identified from chart reviews.</td>
<td>All subjects were interviewed about demographic history, marital and sexual behavior history, and dietary history (method not described). Blood and saliva samples were collected between 9-12 AM (fasting status not indicated). Samples were &quot;kept cool&quot; for between 1-2 hr and then frozen at -20°C until analysis (time frame not given) for retinol, β-carotene, and α-tocopherol. Of the 134 cases of cancer, 107 had blood drawn prior to surgery. Blood from other groups was drawn within days of hospitalization.</td>
<td>Mean levels of serum retinol, β-carotene and α-tocopherol were significantly lower in cases than controls. There was an unadjusted trend of increased risk for prostatic cancer in the combined cases associated with decreasing retinol levels. There was a nonsignificant trend when the clinical cases were analyzed alone. In more severe cases (those who had radical surgery and/or therapy), the significant trend persisted. There was no trend for β-carotene. When adjusted for age and current cigarette smoking, the trend for retinol did not reach significance.</td>
<td>Potential response bias indicated by different numbers of subjects giving blood in different age groups and from admitting hospital vs cooperating hospital referrals. Other potential confounding factors include timing of blood sampling relative to hospital admission, differences in duration of disease, and nonfasted blood samples. No diet or supplement use data were reported.</td>
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<tr>
<td>Hsing et al., 1990a</td>
<td>Case-control, Maryland</td>
<td>103 cases prostate cancer, 103 controls matched for race (all white), age, and sex (all ß) Subjects drawn from a sample pool of 25,802 residents of Washington Co., MD Cases were identified over an 11-yr period (1974-86)</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 30 cases and 30 control samples analyzed at a different lab in a pilot study. The remaining 140 samples (70/70) were analyzed as above. Inter-laboratory variations ranged from 3% for retinol to 11% for β-carotene. None reported for α-tocopherol.</td>
<td>No differences in mean vitamin levels between cases and controls. There was a trend for decreased risk associated with serum retinol levels; no trend associated with either β-carotene or α-tocopherol levels.</td>
<td>No diet or supplement use data reported Long and variable storage time</td>
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| Hsing et al., 1990b   | Cohort/case-control USA           | 149 cases of fatal prostate cancer              | 68.5% of the original cohort completed questionnaires. Comparisons of respondents to nonrespondents showed no differences in age, residence, or policy status. Questionnaire included data on demographics, alcohol and tobacco use, and diet history. Subjects asked about current intake of 35 food items. Portion sizes were estimated from survey data (NHANES II). | No significant trends were associated with total vitamin A, retinol, or β-carotene intake. When analyzed by age the group diagnosed <75 yr had an I risk associated with intake of total vitamin A. In those ≥75 yr the trend was reversed. This pattern held true for retinol and β-carotene. There were no changes in risk associated with intake of any of 9 food groups or any individual foods. | Self-selected population
No comparison with general population, no data on mean intake, no supplement data
The vitamin A differences in the 2 age groups could have reflected a difference in the type of foods eaten; this was not tested.
Very limited food items in food–frequency questionnaire (it lacked some major sources of vitamins, e.g., liver, broccoli, spinach, and melons).
Age analysis was only reported for the vitamin A intake, not for smoking, alcohol consumption, or other foods.
In 56 of the 149 fatalities, prostate cancer was not the primary cause of death. It was not clear whether prostate cancer was the primary diagnosis. |
| Kolonel et al., 1988  | Case-control Hawaii               | 452 cases of histologically-confirmed prostate cancer (PC) 899 age–matched controls. Subjects ≥65 yr were randomly selected from a central insurance registry, those <65 yr selected with random-digit-dialing. | All subjects given an extensive home interview to collect data on dietary, occupational, medical, social, and demographic histories. 100+ item food–frequency questionnaire was used. Reference period was a usual one prior to onset of the disease for cases and a corresponding period for controls. Surrogates were used for those subjects who could not be interviewed. | Older cases consumed significantly more saturated fat, total vitamin A, and zinc than age–matched controls. These differences were reflected in increased risk associated with saturated fat and zinc. There was a significant increase in risk with the highest quartile of total vitamin A intake as well as a trend towards increased risk. Similar finding with respect to total carotenes and β-carotene. No difference between younger subjects and their matched controls. There were no associations between risk and total vitamin C or food sources of vitamin C. No differences found in potential confounding variables: SES, marital status, anthropometries, family history No significant interactions between nutrients | Total vitamin A and total zinc included supplements. The supplements were not characterized as either individual or multivitamins/minerals. At the time of the study β-carotene was not available in Hawaii as a supplement. The older cases consumed more of all forms of vitamin A than younger cases with the exception of food sources of retinol. This would indicate a greater use of supplements or greater intakes of carotene-rich foods. The mean weekly intake of total vitamin A was 12,657 IU in the older group vs 11,028 IU in the younger cases. These intake levels are both >2x the RDA (8000 of 5000 IU/d for adult). The duration of supplement use was not reported. |
| Le Marchand et al., 1981 | Case-control Hawaii               | See Kolonel et al., 1988                        | See Kolonel et al., 1988 Data set was analyzed separately for <70 and ≥70 yr | Main food sources of β-carotene were carrots, papaya, pumpkin, sweet potatoes, and mangoes. In <70 yr group there was no association between risk and intake of these foods. In older group there was a strong association with papaya only. | There were no differences reported in mean intake of β-carotene rich foods between the older and younger groups. β-carotene from papaya is an unlikely cause of prostate cancer.
No analysis of food and supplement separately or in interactions although at the time of the study β-carotene was not available as a supplement.
Total vitamin A intake included supplements. |
### APPENDIX TABLE. VITAMIN A, CAROTENOIDs, AND CANCER (Observational Studies x Site)

<table>
<thead>
<tr>
<th>Study</th>
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<th>Subject # &amp; Description</th>
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<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mettlin et al., 1989</td>
<td>Case-control</td>
<td>371 cases of histologically confirmed prostate cancer</td>
<td>All patients admitted to the Roswell Park Memorial Institute are given a</td>
<td>No differences in age, marital status, education, weight, and height. There was a geographical difference. There was a significant reduction in risk associated with the highest level of intake of β-carotene in ♂ &lt;68 yr but not in subjects &gt;68 yr. Age-</td>
<td>No supplement data, no population-based controls. Diet data collected during time of duress for most subjects. Variable time between first diagnosis and hospital admission.</td>
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<td>Buffalo, NY</td>
<td>371 control patients with no history of cancer, matched by age</td>
<td>lifestyle questionnaire including a 40-item food-frequency checklist.</td>
<td>and resident-adjusted risk for highest level of β-carotene for the combined age groups shows a protective effect. Increased consumption of high fat milk was associated with increased risk. There was a nonsignificant trend towards increased risk associated with fat intake.</td>
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<td>12.1% of controls had benign prostatic hyperplasia</td>
<td>Reference period for all patients was the period preceding the onset of current illness (admission?). Portion sizes were estimated from standard food tables.</td>
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<td>There were a total of 76 different diseases in this group.</td>
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<tr>
<td>Ohno et al., 1988</td>
<td>Case-control</td>
<td>100 cases newly diagnosed of prostatic cancer (PC)</td>
<td>Data collected by interview upon admission to hospital included:</td>
<td>Low intakes of vitamin A (retinol and β-carotene) were associated with increased risk. The risk reduction associated with vitamin A and β-carotene was seen in older (70–79 yr) but not younger (50–69 yr) ♂. Vitamin A and β-carotene from green/yellow vegetables were significantly protective. There was no association between risk and any other nutrients.</td>
<td>No supplement data, confusing statistics, lack of community-based controls. Long retrospective period, 5 yr. No smoking data.</td>
</tr>
<tr>
<td>Ouishi et al., 1988</td>
<td>Japan</td>
<td>100 controls with benign prostatic hyperplasia (BPH)</td>
<td>birthplace, occupational history, marital history, religion, body type,</td>
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<td>100 hospital controls without BPH, other malignancies, liver disease, or hormonal disorder</td>
<td>medical history, sex–life, and dietary practices. Food–frequency questionnaire assessed dietary habits during the period 5 yr prior to current admission. Photographs were used to estimate portion sizes.</td>
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<td>All subjects were matched for hospital, age (± 3 yr) and data of admission (± 3 mo).</td>
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<td>Reichman et al., 1990</td>
<td>Cohort/case-control</td>
<td>84 cases of prostate cancer</td>
<td>NHEFS collected health outcome and dietary data on subjects who were &gt;25 yr at time of NHANES I. The study cohort consisted of those subjects who agreed to follow-up and had blood samples taken during NHANES I. Serum samples were collected and stored at -20°C for a period &lt;3 mo.</td>
<td>There was a higher percentage of blacks than whites in the lower percentiles of vitamin A levels. Education, serum cholesterol, alcohol consumption, and body-mass index were all associated with vitamin A levels. Age, marital status, height, total calories, and smoking were not associated with vitamin A in this cohort. There was a statistically significant difference in mean vitamin A levels between cases and controls. There was a significant negative trend for risk of prostate cancer associated with vitamin A levels. There was no difference in risk in those over 70 yr.</td>
<td>No diet interactions tested. Supplement use was noted to be related to vitamin A levels; however, supplements were not characterized. No distinction made between forms of vitamin A; presumably methods used analyzed total vitamin A content.</td>
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<tr>
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| Ross et al., 1987 | Case–control California | 179 black (BPC) of prostate cancer (PC) diagnosed between 1977–80  
142 black controls (BC) matched for age (± 5 y) and residence  
142 white (WPC) of PC diagnosed between 1972–1982  
142 white controls (WC) | All interviews usually done at home (all WPC and WC) or occasionally at an mutually–convenient location.  
A food–frequency questionnaire containing 20 categories of foods was used to estimate intake of fat, protein, or vitamin A. Reference period was time of diagnosis  
Portions estimated from common portion sizes | Significant differences between races for sexual practices and incidence of venereal disease.  
Venereal disease (+) and circumcision (−) were significantly associated with risk in both groups.  
Vitamin A consumption was inconsistently related or unrelated to PC risk in both groups.  
Fat intake was a risk factor for both groups. | 57% response rate for black cases  
Variable and long reference period  
Limited items on food–frequency questionnaire  
Portion sizes estimated from food tables  
No supplement use data  
Groups differed demographically  
BC were apparently not matched to BPC group  
Demographically  
There were no statistical adjustments made for any confounding variables in the diet analysis. |
| Severson et al., 1989 | Cohort/ case–control Hawaii | 174 cases of newly diagnosed malignant prostate cancer divided into overt PC (OPC) and latent cancer (LPC)  
Cohort consisted of 7999 of Japanese ancestry | All subjects interviewed between 1965–1968 about demographics, marital, smoking, occupational, residence, education, alcohol use, and medical history.  
23–item food–frequency questionnaire and 24–hr recall Reference period was time of initial examination to time of diagnosis. | Individual nutrients not evaluated  
No relationship between intake of total fat and protein  
Intake of certain types of foods, e.g., seaweed (+) and rice (−), were associated with risk. | Reference period unclear and variable  
No supplement use data; no biochemistry  
Limited nutrient data  
Not designed to assess vitamin A or any other specific nutrient  
No comparison to general population, limited to traditional Japanese–type diet |
### APPENDIX TABLE: VITAMIN A, CAROTENOIDS, AND CANCER (Observational Studies x Site)

#### X. Bladder Cancer

<table>
<thead>
<tr>
<th>Study</th>
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<tr>
<td>Helzlsouer et al., 1989</td>
<td>Case-control Maryland</td>
<td>35 cases of bladder cancer; 70 controls (2/case) matched for nearest age, sex, race within 2 hr interval of blood sampling and last meal; Sample pool was 20,305 residents of Washington Co., MD; Cases were identified over an 11-yr period (1975-86).</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 and lycopene levels were nonsignificantly 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.</td>
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<td>La Vecchia et al., 1989</td>
<td>Case-control northern Italy</td>
<td>163 cases of histologically confirmed (within 1 yr before interview) bladder cancer (total pool eligible not given); 181 hospital controls (HC)</td>
<td>All subjects interviewed about SES factors, smoking, alcohol, coffee, and other methylnitrosoamine consumption habits, personal and family health history, and specific medication history. Frequency of consumption of 10 food items. Reference period for cases was the period before onset of symptoms; none was given for controls.</td>
<td>The frequency of consumption of green vegetables and carrots was significantly lower in cases. Estimated intakes of carotenoids and total vitamin A, but not retinoids, were significantly less in cases than controls. There was increased risk for BC associated with estimated low intakes of both carotenoids and retinol. Protective effect was stronger in current smokers. No effect from either fruit or vitamin C.</td>
<td>Reference period was at least 2 yr before interview for cases. Very limited number of items (10) on food–frequency questionnaire. No supplement use data. No community–based controls. Portion size was estimated by investigators.</td>
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<tr>
<td>Steineck et al., 1990</td>
<td>Case-control Stockholm, Sweden</td>
<td>418 cases of urothelial cancer and or squamous cell cancer of the lower urinary tract (renal pelvis, bladder, ureter, urethra); 511 sex- and age-stratified, randomly-selected controls</td>
<td>Questionnaire was mailed to all subjects and included health history, drug use, occupation, smoking, &quot;life events&quot;, and diet. 55–item food–frequency section. Reference period was 3 yr prior to interview. Portions were estimated with photographs. Separate questions about supplement use; specifically, vitamins A, B, and C and &quot;other kinds of supplements and tonics.&quot; Study period was 1985-1987, supplement use data after 1981 was ignored.</td>
<td>Supplemental intake of vitamin A (uncharacterized) was inversely associated with risk. Fat and fried foods were significantly associated with increased risk.</td>
<td>The nature of the collection of the dietary data set was not clearly delineated. Reference period was confusing. An apparently long retrospective period between supplement use reference period and study interview. Vitamin supplements broadly categorized, e.g., vitamin A or vitamin B.</td>
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<td>Greenberg et al., 1990</td>
<td>Intervention/ randomized double-blind trial 4 clinical centers: Dartmouth, UCLA, UC-San Francisco, Univ. Minnesota</td>
<td>Potential subjects were patients with non-melanoma skin cancer (basal-cell or squamous cell carcinoma) since 1980. 1805 subjects were selected. Subjects were randomized to receive 50 mg/d β-carotene or placebo. At 4-mo intervals subjects responded to a questionnaire about health, compliance, consumption of vegetables, and use of vitamins. Subjects returned annually for examination or sent detailed record of annual examination. Blood samples were collected at enrollment and annually thereafter. Plasma shipped at -20°C and subsequently stored at -70°C until assayed for β-carotene and retinol.</td>
<td>Subjects were randomized to receive 50 mg/d β-carotene or placebo. At 4-mo intervals subjects responded to a questionnaire about health, compliance, consumption of vegetables, and use of vitamins. Subjects returned annually for examination or sent detailed record of annual examination. Blood samples were collected at enrollment and annually thereafter. Plasma shipped at -20°C and subsequently stored at -70°C until assayed for β-carotene and retinol.</td>
<td>No difference in occurrence of new non-melanoma skin cancer between groups</td>
<td>38% of eligible subjects agreed to participate. No characterization of baseline or concurrent dietary habits</td>
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<td>Stryker et al., 1990</td>
<td>Case-control study Boston</td>
<td>204 cases of malignant melanoma 248 control patients who were making first visit to clinic 204 cases of malignant melanoma 248 control patients who were making first visit to clinic 204 cases of malignant melanoma 248 control patients who were making first visit to clinic 204 cases of malignant melanoma 248 control patients who were making first visit to clinic</td>
<td>All subjects completed a questionnaire on diet (food frequency for 116 foods, questions on use of vitamin and mineral supplements and on type of fats used for cooking) medical history, and constitutional and lifestyle factors. Other factors collected included demographic data, pigmentation characteristics, and past medical history. Fasting serum samples stored at -70°C for up to 6 mo. Subjects estimated portion sizes. Reference period was the previous yr.</td>
<td>All subjects completed a questionnaire on diet (food frequency for 116 foods, questions on use of vitamin and mineral supplements and on type of fats used for cooking) medical history, and constitutional and lifestyle factors. Other factors collected included demographic data, pigmentation characteristics, and past medical history. Fasting serum samples stored at -70°C for up to 6 mo. Subjects estimated portion sizes. Reference period was the previous yr.</td>
<td>Cases were more likely to be supplement users than controls. After adjustment for age, sex, plasma lipids, hair color, and tanning ability, levels of lycopene, retinol, and α-tocopherol were similar in cases and controls. Controls tended to have a greater intake of caroten and dietary vitamin E. No differences in descriptive or risk-trend analyses between groups for plasma caroten (α-, β-, or lycopene) or retinol. There was a weak non-significant trend towards a lower intake associated with a carotene intake. There was a trend towards a lower intake of vegetable fat. Some of the higher levels of α-tocopherol were associated with a nonsignificant decreased risk of melanoma. Intake of vitamin E from food alone was significantly associated with a trend of decreased risk with increased intake.</td>
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## XII. Other Studies

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<tr>
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| Knekt et al., 1990     | Case-control   | 766 cases of cancer of all sites  
1,419 controls matched for age, sex, and duration of storage  
Subjects drawn from a pool of 36,263 survey participants  
Cases identified over a variable time period of 5-9 yr | Baseline questionnaire included data about occupation, drug use, medical history, and smoking habits.  
Body-mass index was used to describe obesity.  
Serum samples were stored at -20°C for between 11-15 yr before analysis of retinol, RBP, β-carotene, selenium, and α-tocopherol. | Mean β-carotene levels were significantly inversely associated with smoking.  
<sup>σ</sup> cases had significantly lower levels of retinol, RBP, and β-carotene than <sup>σ</sup> controls.  
<sup>σ</sup> cases had nonsignificantly lower levels of all vitamin A-related compounds.  
Among <sup>σ</sup> there was a significant inverse relationship between retinol and β-carotene and risk after adjustment for smoking.  
There was an inverse gradient between serum retinol levels and the occurrence of cancer in <sup>σ</sup> that was primarily concentrated in the first 2 yr of follow-up.  
The differences in β-carotene were reflected in risk of lung cancer.  
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.  
<sup>σ</sup> with low vitamin E and low Se had a 3x higher risk of hormone-related cancer. | No diet data, supplement use data, or matching for SES  
No seasonal variations in food supply and time of blood sampling reported  
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. |
| Paganini-Hill et al., 1987 | Cohort           | Cohort consisted of 10,437 residents of a retirement community in CA.  
All subjects were free of preexisting cancers.  
Subjects were primarily white, moderately affluent and well-educated.  
Median age = 74 yr, 67% <sup>σ</sup>. | Subjects were mailed a questionnaire about demographics, medical history, personal habits, medical screening use, diet, and (for <sup>σ</sup>) menstrual and reproductive history.  
Diet data included vitamin supplement use and a 59-item food-frequency questionnaire.  
Portion sizes were estimated using standard tables.  
Original questionnaire sent in 1981 followed by biennial questionnaires in 1983 and 1985. | By 1986, 643 cases of cancer:  
58 lung, 110 colon, 59 bladder,  
93 prostate, 123 <sup>σ</sup> breast cancer, 202 cancers of other sites.  
Neither vitamin or β-carotene had a statistically significant effect on overall cancer rates.  
For the sites considered, there was a significant trend for 1 risk for bladder cancer in <sup>σ</sup> with increased consumption of dietary β-carotene.  
Dietary intake of vitamin A was inversely associated with smoking.  
The incidence rate for all cancers in <sup>σ</sup> who never smoked was inversely related to β-carotene and total vitamin A intakes. | Selective cohort of mid-high SES and health conscious  
No case-control comparisons  
Supplements used not characterised  
Diet data of questionable reliability  
There was only a 50% agreement between the questionnaire used in the first and second follow-up. |
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<tr>
<td>Stähelin et al., 1987</td>
<td>Prospective cohort</td>
<td>Basel, Switzerland</td>
<td>2974 # comprised the original pool from which 304 total cancer deaths occurred: 68 lung cancer, 20 stomach cancer, 17 colorectal, and 99 &quot;other malignancies&quot;.</td>
<td>Compared to survivors, cases had significantly lower plasma carotene levels. Carotene levels were significantly lower in lung and stomach cancer cases. Vitamin C was also lower in all cancer cases than in controls. Low carotene levels were associated with 1 risk for lung cancer after adjustment for smoking, age, and cholesterol. For all cancers the combination of low carotene and low vitamin A was associated with 1 risk. After adjustment for smoking, vitamin C levels were significantly lower in stomach cancer deaths.</td>
<td>No diet data and no supplement data to control for seasonal variations in diet that might influence blood levels Reliance on point sample procedures</td>
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