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NUTRITION AND CANCER PREVENTION: A Multidisciplinary Perspective on Human Trials*

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■ **Abstract** More than one million Americans were expected to be diagnosed with cancer in 2003 (7a). Compelling experimental, epidemiological, and clinical evidence indicates that many cancers are preventable, especially because diet and nutrition are key factors in the modulation of cancer risk. The road to nutritional intervention in cancer prevention has led to successful trials as well as trials that did not reach their intended endpoints. This chapter reviews four case studies of trials, with two ending in success and two ending in null findings or adverse effects. The goal is to identify lessons learned from all four case studies and from the investigations of the complexities inherent to nutritional intervention trials. Additional insights are presented by the research addressing potential mechanisms underlying the endpoints of human trials. Future progress in nutrition and cancer prevention will require expertise from multidisciplinary teams to develop new knowledge about specific nutrients and dietary modifications within a framework of interaction between animal and human research.

CONTENTS

INTRODUCTION

Cancer is preventable. The *1964 Surgeon General's Report on Smoking and Health* identified cigarette smoking as the primary cause of lung cancer (1). Since the Surgeon General's Report was published, declines in lung cancer deaths in white men since 1991 and in black men since 1993 reflect reductions in tobacco use, with several effective community-based interventions that include regulations to restrict smoking in public places and tobacco product advertising to adolescents and children (95b). Few nonsmokers suffer from lung cancer, demonstrating the optimal preventive behavior. Perhaps the earliest and most successful example of nutrition intervention in the United States (90) was Goldberger et al.'s effort to eliminate pellagra—a disease associated with a diet deficient in niacin—through dietary modifications in high-risk communities in the early 1900s. The strongest epidemiologic determinant of cancer risk—after smoking—is diet. Thus, a better understanding of nutrition and cancer prevention could have a major public health impact. When we fast-forward to recent randomized controlled trials, many successes appear in the field of nutritional prevention of cancer; however, other trials reveal null findings and even unsuspected adverse effects. Further examination of the successes and failures in nutritional interventions is critical.

The era of human nutrition trials in cancer prevention began in 1981 with the seminal papers by Peto (71, 72), who examined the role of β-carotene and vitamin A in cancer prevention. This period of research focused on the effect of a single agent intervention in a model of human carcinogenesis directed toward identification of inhibitors of late-stage disease. Since 1981, there have been numerous trials of retinoids, carotenoids, and combinations of pharmacologic doses of nutritional agents. Trials are costly and long because of the time required to reach cancer endpoints in a sufficient number of participants, and compliance—related

to the intervention and to the screening procedure for endpoint ascertainment—may not be optimal. During the long period of an intervention trial, new observational epidemiological and animal research may identify alternative interventions and suggest other approaches for prevention. Changing secular trends in human behaviors and exposures can also complicate the interpretation of trial results.

Randomized controlled trials are the gold standard to test cause and effect in human research. Ethical considerations limit the genre to the effect of dietary or other lifestyle interventions, such as physical activity, and preventive agents on an endpoint. The distribution of risk factors for cancer, typically the domain of observational epidemiology, may not include a sufficient range of the exposure to identify the optimal level or dose to prevent disease, depending on the populations studied (60). Therefore, before conducting a randomized clinical trial of nutritional prevention of cancer, we turn to animal experiments and clinical nutrition research to fill in the gaps in our understanding of the etiologic mechanisms underlying the exposure-disease association and in clarifying the metabolic effects of a "nutritional dose." With the advent of molecular-based targeted interventions, the steps in carcinogenesis can be identified prior to a trial and can lead to alternative approaches to prevention models.

In this chapter, we briefly review multistage carcinogenesis and identify targets for prevention. Four case studies of human nutritional trials in cancer prevention are examined in an effort to understand why two sets of case studies did not achieve the expected cancer preventive outcomes and the other two reached their goals of reducing recurrence of a late intermediate endpoint for colorectal cancer or prevented second primary cancers. Our presentation first describes the state of the art prior to each series of trials, followed by a discussion of the trial design, selection of the preventive agent or dietary intervention studied, determination of the primary endpoints, analysis of the results, and recommendations for directions for future research. Reflections on the results of case studies are threaded with investigations of the complexities inherent to nutritional intervention trials and mechanisms underlying the adverse or null endpoints. Finally, using lessons learned from the trials, we identify achievable goals, as well as the underpinnings to and the relevant criteria for future human research in the nutritional prevention of cancer.

Methods

Human trials of nutritional prevention of cancer were selected from a series of endpoints and of interventions. Specifically, using the Medline and other search engines, references were selected to illustrate three endpoints, i.e., primary endpoints of cancer incidence and of second primary cancers, as well as late intermediate biomarkers of cancer. Three types of interventions were selected: dietary modulations by whole diet or bioactive components in diet, vitamin/mineral supplementation, and pharmacologic doses of naturally occurring substances or synthetics. The criteria for selection of the specific case series were formulated to

illustrate the larger domain of nutrition prevention trials in an area and to elucidate multidisciplinary creative approaches, identify gaps in the field as well as explore strategies for future interventions.

MULTISTAGE CARCINOGENESIS PROCESS AND TARGETS FOR PREVENTION

Humans are exposed to a wide variety of endogenous and exogenous carcinogenic insults, including chemicals, radiation, physical agents, bacteria, and viruses. Recent progress in the carcinogenesis field, particularly on the mechanisms of chemically and virally induced cancer, has revealed several points along the carcinogenesis pathway that may be amenable to cancer prevention strategies. The classic view of experimental carcinogenesis (19a, 79a), in which tumor initiation is followed by tumor promotion and progression in a sequential fashion, has undergone significant revision as our understanding of cancer-related genes and the biosystem has evolved. However, the concepts and underlying processes of initiation, promotion, and progression remain theoretically important. Tumor initiation begins in cells with DNA damage resulting from inherent genetic alterations or, more commonly, from spontaneous or carcinogen-induced genetic or epigenetic alterations (31, 95b). Alterations in specific genes modify the responsiveness of the initiated cell to its microenvironment, eventually providing a growth advantage relative to normal cells. The tumor promotion stage is characterized by clonal expansion of initiated cells due to alterations in the expression of genes whose products are associated with hyperproliferation, apoptosis, tissue remodeling, and inflammation (79). During the tumor progression stage, preneoplastic cells develop into invasive tumors through further clonal expansion, usually associated with alterations in gene expression and additional genetic damage due to progressive genomic instability (73).

As depicted in Figure 1, possible ways of interfering with tumor initiation events include (*a*) modifying carcinogen activation by inhibiting the enzymes responsible for that activation or by directly scavenging DNA-reactive electrophiles and free radicals; (*b*) enhancing carcinogen detoxification by altering the activity of detoxifying enzymes; and (*c*) modulating certain DNA repair processes. Possible ways of blocking the processes involved in the promotion and progression stages of carcinogenesis include (*a*) scavenging reactive oxygen species; (*b*) altering the expression of genes involved in cell signaling, particularly those regulating cell proliferation, apoptosis, and differentiation; (*c*) decreasing inflammation; (*d*) enhancing immune function; or (*e*) suppressing angiogenesis (12a).

In 1976, Sporn defined the term chemoprevention as the use of natural or synthetic agents to reverse or suppress multistage carcinogenesis (84, 85). Numerous examples in the literature demonstrate that bioactive food components or chemopreventive nutrients can influence one or more of the targets described above and interfere with the carcinogenesis process (31).

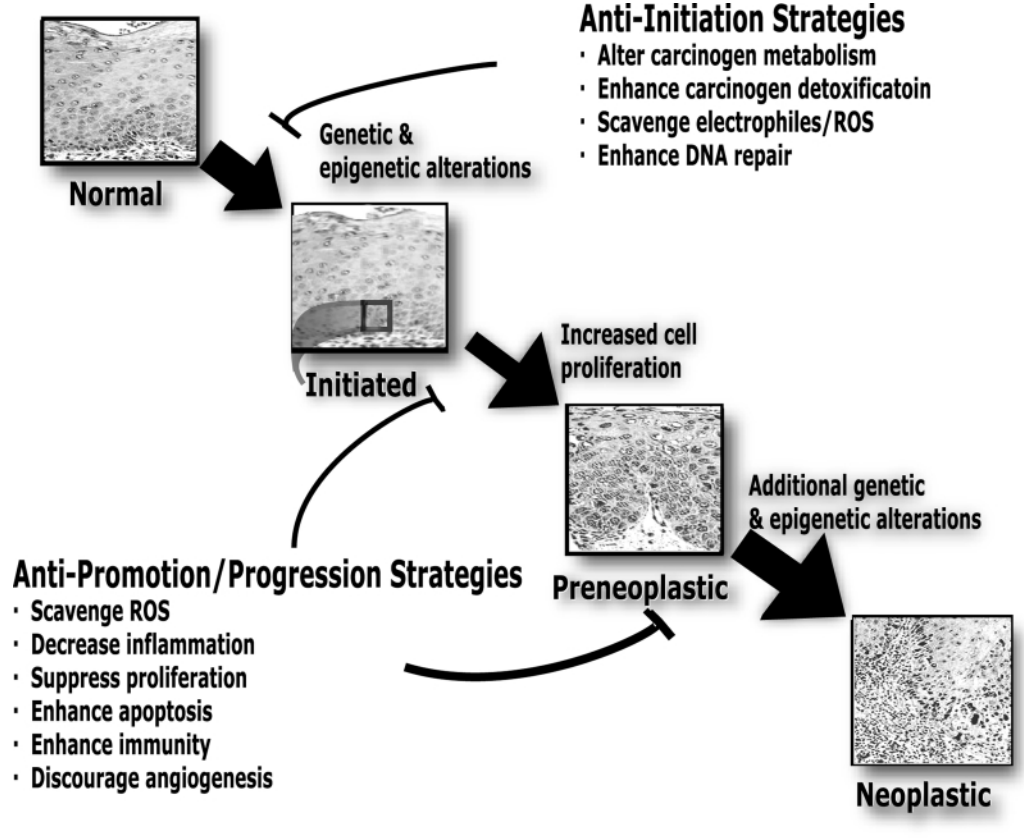

Figure 1 Multistage carcinogenesis model.

We now appreciate that the nature of initiation, promotion, and progression of events is complex. For instance, we know from the work of Vogelstein and others that multiple mutational and epigenetic events are involved in the formation of cancers (19). Furthermore, humans are generally exposed to mixtures of agents that can simultaneously act at different stages of the carcinogenesis process. Thus, rather than three discrete stages occurring in a predictable order, human carcinogenesis is best characterized as an accumulation of alterations in genes regulating cellular growth, death, and malignant properties. These alterations occur through a series of clonal selections influenced by endogenous and exogenous factors. Concomitant epigenetic instabilities often develop in a cancer and may also drive this multistep process (86). Nonetheless, the processes involved in cancer initiation, promotion, and progression, described above, remain important and relevant targets for cancer prevention.

CASE SERIES I: TRIALS OF β-CAROTENE SUPPLEMENTATION AND LUNG CANCER INCIDENCE

In this section, we examine four trials of β-carotene supplementation that led to increased risk of or had no effects on lung cancer incidence, and we describe the research that explores potential underlying mechanisms for the enhancement of lung cancer in the β-carotene supplementation arm. For many years prior to the trials, qualitative differences in uptake and absorption of β-carotene and vitamin A were reported in pharmacokinetic studies in humans and rodents (21, 30). Indeed, the distinctive pharmacokinetics of retinol and β-carotene led Peto et al. (72) in 1981 to stipulate that their chemopreventive effects should be assessed separately. In the oft-cited paper entitled "Can dietary β-carotene materially reduce human cancer rates?" Peto (71) discussed (*a*) the role of retinol and the retinoids in later-stage carcinogenesis and the absence of experimental evidence for β-carotene; (*b*) epidemiologic data that did not constitute "overwhelming" evidence for any real chemopreventive effects from serum concentrations of retinol or β-carotene; and (*c*) speculation by Peto et al. that "people who eat lots of vegetables may get β-carotene and other truly protective factors from them or refrain from eating a harmful dietary component" (72). Finally, Peto et al. recommended β-carotene, rather than vitamin A, chemoprevention trials in the general population aged $\geq$50 because high doses of β-carotene for treatment of erythropoietic protoporphyia were not associated with toxicity, as was the case for vitamin A (56, 72).

β-Carotene Supplementation Trials of Lung Cancer

Following publication of this paper, four large-scale randomized placebo-controlled trials of β-carotene were initiated. The results revealed adverse effects or no difference in rates of lung cancer among participants in the β-carotene arm compared to the placebo arm (see Table 1). Two trials enrolled high-risk individuals while

TABLE 1 Lung cancer prevention trials evaluating beta-carotene supplementation

Trial	Agent(s)	Population	N	F/U	Risk effect
ATBC (Finland)	β-carotene (20 mg/d) Vitamin E (50 mg/d) (2×2 design)	Male smokers 50–69 yr ($\mu = 57$ yr) 36 years of smoking	29133	5–8 yr $\mu = 6$ yr	876 cases RR = 1.18 (1.03, 1.36)
CARET (United States)	β-carotene (30 mg/d) Retinyl palmitate (25,000 IU) (2×2 design)	Men and women smokers ($\mu = 58$ yr) 49 years of smoking asbestos ($\mu = 57$ yr) 43 years of smoking	18314 14254 4060	4–7 yr $\mu = 4$ yr	286 cases RR = 1.36 (1.07–1.73) β-carotenesuppl. RR = 1.28 (1.04, 1.57) β-car+retinyl pal. RR = 1.40 (0.95–2.07) asbestos exposed RR = 1.23 (0.96–1.56) heavy smokers
PHS I (United States)	β-carotene (50 mg/alternate/d)	Male MDs age 40–84 11% smokers	22071	12 yr	82 cases in β-carotene 88 cases in placebo RR = 0.98 (0.91, 1.06)
WHS (United States)	β-carotene (50 mg/alternate/d) of 8 groups	Women age 45 + 13% smokers	39876	2.1 yr	30 cases in β-carotene 21 in placebo

Abbreviations: ATBC, Alpha-Tocopherol β-Carotene Trial; CARET, Carotenoid and Retinol Efficacy Trial; F/U, years of follow-up during the trial; N, number randomized; PHS, Physicians' Health Study; RR, relative risk; WHS, Women's Health Study

Note: All trials were designed as randomized controlled trials

the other two enrolled apparently healthy individuals. The Alpha-Tocopherol β-Carotene (ATBC) Trial in Finland included male smokers at high risk of lung cancer (mean = 36 years smoking) who were administered β-carotene, α-tocopherol, both, or a placebo for six years on average. The β-Carotene Retinol Efficacy Trial (CARET) in the United States enrolled men and women who were either smokers (mean = 49 years) or asbestos-exposed workers, and administered β-carotene, retinyl palmitate, both, or a placebo for four years on average. In these two trials, the β-carotene-supplemented group experienced an increased risk of lung cancer (by 18% in ATBC and 36% in CARET) compared with the placebo group, and the effect appeared stronger in participants who drank higher-than-average amounts of alcohol daily (i.e., a 35% versus 3% increase in lung cancer risk, respectively, in ATBC) (4, 7, 67). Also, more of a systemic effect appeared in subsequent analysis as illustrated by a 23% and 25% increase in prostate and in stomach cancers, respectively, in the supplemented (versus placebo) group in ATBC and an 18% and 26% increase in total mortality and in cardiovascular deaths among the β-carotene group in CARET. In contrast, participants in the Physicians Health Trial and the Women's Health Study were apparently healthy, had a low rate of smokers, and were administered an alternate day schedule of β-carotene for follow-up of 12 and 2 years, respectively (22, 38). Compared with the placebo group, β-carotene supplementation in these participants was not associated with an increased risk of lung cancer, and indeed rates of lung cancer did not differ by treatment arm. In all four trials, participants in the β-carotene-supplemented arm compared with the placebo arm had significantly higher concentrations of β-carotene in the blood, indicating uptake of the supplement, but not necessarily efficacy.

Our search for reasons why β-carotene supplementation was associated with adverse effects focused on several arenas that revealed inconsistent epidemiological results, limited animal experiments, and insufficient nutrition research.

EVIDENCE FROM OBSERVATIONAL EPIDEMIOLOGY A review of epidemiological studies (35) covering the period before, during, and soon after the four trials revealed more significant inverse associations between serum concentrations and dietary intake of β-carotene and lung cancer in case-control than in cohort studies. In addition, although for many years, algorithms were available to estimate β-carotene intake from fruits and vegetables, no overwhelming evidence was found for an inverse association between intake of fruits and vegetables and risk of lung cancer (35). Of note, dietary intake of β-carotene was estimated from two different databases over time. Specifically, the World Health Organization/Food and Agriculture Organization algorithm for provitamin A intake preceded the 1993 National Cancer Institute/U.S. Department of Agriculture (USDA) Carotenoid Food Composition Data Base (51). Two different laboratory procedures for detection of β-carotene in the blood were available, notably the earlier use of spectrophotometric chromatography and the later use of high-performance liquid chromatography. These methodological differences in estimation of intake and blood values may have complicated comparisons and potentially contributed to inconsistent results.

Results of research on serum retinol concentrations as well as on dietary vitamin A intake and lung cancer were less consistent than the results of serum β-carotene–lung cancer research across cohort and case-control studies (35). Thus, the pattern of consistent inverse associations between serum levels and dietary intake of β-carotene and lung cancer only appeared in case-control, not cohort, studies over time, yet results from cohort studies avoid the problems of inaccurate reporting of diet and recall bias.

EVIDENCE FROM ANIMAL RESEARCH A few experimental studies had been conducted to examine the effect of β-carotene on skin cancer before the start of randomized clinical trials in humans. Two research groups subsequently addressed major questions arising from the trials. The USDA-Tufts group focused on research to explain the "apparent exacerbation of lung carcinogenesis by β-carotene supplementation in smokers" (94). In a six-month study of four groups of ferrets—the first given β-carotene supplementation, the second exposed to cigarette smoke, the third receiving β-carotene and exposure to smoke, and the fourth group receiving neither β-carotene nor exposure to smoke—a strong proliferative response in lung tissue and squamous metaplasia was observed in the β-carotene-supplemented animals and especially enhanced in the group exposed to cigarette smoke. With in vitro incubation of all-*trans*-β-carotene in lung tissue from either smoke-exposed or nonexposed ferrets, the β-carotene molecule is unstable and forms oxidative byproducts that induce cytochrome P450 enzymes and interfere with retinoic acid metabolism as well as down-regulate retinoic acid receptor (RAR)β (93). Indeed, the down-regulation of RARβ expression in the lungs of ferrets who were receiving both β-carotene supplementation and smoke exposure could be the mechanism for enhancement of lung tumorogenesis.

In a recent follow-up study, ferrets were either exposed to a smoking chamber at levels of cigarette smoke comparable to 1–2 packs/day, or not exposed to smoke, and administered supplements of another carotenoid, lycopene, in 15- or 60-mg/d doses for nine weeks (47). Compared with ferrets exposed to smoke alone, ferrets supplemented with lycopene and exposed to smoke had significantly higher plasma insulin-like growth factor–binding protein 3 (IGFBP-3) and a lower insulin-like growth factor-1 (IGF-1)/IGFBP-3 ratio, thereby reducing serum levels of bioavailable IGF-1. Both low- and high-dose lycopene supplementation inhibited lung squamous metaplasia in a dose-dependent fashion among the smoke-exposed ferrets (47). Cigarette smoke exposure increased BAD [a proapoptotic Bcl-2 family protein homology (BH) domain on BH3] phosphorylation and significantly decreased cleaved caspase-3 in the lungs of ferrets; however, lycopene supplementation prevented the elevated phosphorylation of BAD and downregulation of apoptosis. This recent study begins to unravel the mystery of the carotenoid lycopene, its potential chemopreventive capacity, and the underlying mechanisms of the carotenoid–lung cancer effects or lack thereof observed in human trials. Finally, the Veterans Hospital group described hepatotoxicity from the interaction of alcohol with β-carotene in baboons (40), thereby demonstrating adverse effects

from exposure to alcohol in the presence of β-carotene as observed in the ATBC (5). Both sets of animal experiments reveal the potential toxic effects of pharmaceutical doses of apparently nontoxic bioactive dietary forms of β-carotene with high-risk behaviors of smoking and alcohol intake.

One of the proposed mechanisms for β-carotene is an antioxidant. Antioxidants can both reduce and accelerate the carcinogenesis process (53). For example, using a well-established antioxidant (N-acetyl-L-cysteine-NAC), Martin et al. (54) showed a 43% reduction of total skin cancers in a mouse model, but a 25% increase in malignant cancers.

EVIDENCE FROM PHARMACOKINETIC AND PHARMACODYNAMIC RESEARCH Prior to the β-carotene trials, limited pharmacokinetic research was conducted, and an optimal dose and the necessary duration of supplementation were not determined. Single-dose studies of β-carotene that demonstrated peak plasma response within 24–48 hours were conducted in healthy young men before the trials (12). Chronic dose studies, ranging from 15–180 mg/d of β-carotene, were conducted in healthy participants at the same time as the trials (16, 57, 62). The chronic dose studies began to reveal large inter-individual variation in response to the varying dosages. Along with limited information on the bioavailability of β-carotene was the issue of distinguishing responders from nonresponders, a phenomenon later reported in trial participants (5). Human nutrition research was not conducted to test the effects of β-carotene supplementation in high-risk groups, such as smokers and drinkers of alcohol in the ATBC and CARET studies. The publication of adverse effects in human trials led to the conclusion that animal research was the only ethical approach to examine the interaction of multiple risk behaviors with a pharmaceutical dose nutrient supplementation and to understand the complex molecular pathways leading to lung carcinogenesis.

CASE SERIES II: HIGH FIBER, FRUIT AND VEGETABLE INTERVENTIONS, AND POLYP RECURRENCE

In this section, we review the strong lines of evidence available before dietary intervention trials were conducted to modify fat, fiber, and fruit-vegetable intake in individuals who had a colon polyp removed prior to the trial. Trial participants were followed over several years without a difference in polyp recurrence in the intervention arm compared with those on their usual dietary regimen. During the 1980s, three lines of evidence suggested that certain aspects of lifestyle, especially dietary factors, were associated with colon carcinogenesis. First, mortality rates for colorectal cancer varied by region of the world. There was a large variation in worldwide rates of colorectal cancer. The second line of evidence appeared in the rapid changes in colorectal cancer incidence within a country. Over a 40-year period in Japan, for example, colorectal cancer incidence rose at a dramatic rate in men more than in women. The third line of evidence came from migration

studies demonstrating that risk of colorectal cancer (CRC) changed with adoption of a new diet. For example, the CRC incidence rate for American-born Japanese was higher than the rate for native Japanese (48). Indeed in most cases, migrants had acquired the CRC rates of their new country within a generation. Moreover, animal evidence overwhelmingly revealed a protective effect of fiber on colon carcinogenesis (74–76). Thus, the 1988 *Surgeon General's Report on Nutrition: Diet and Colorectal Cancer (CRC) Risk* (92a) and the 1989 *National Academy of Sciences Diet and Health Report* (63a) summarized the field by concluding there was sufficient evidence that a high-fat diet increased CRC risk, and a diet high in fiber, fruits, and vegetables decreased CRC risk.

During this era, the progression of colorectal carcinogenesis was depicted as a series of sequential steps from normal epithelium to development of aberrant crypts, followed by early adenomatous polyps, the formation of advanced adenomas, and finally cancer. Population-based incidence rates of adenomatous polyps were not available, and identification of a high-risk group other than those with familial adenomatous polyposis syndrome was difficult. Therefore, investigators in cancer prevention focused on trials of dietary interventions to reduce recurrence of adenomatous polyps as a viable late intermediate endpoint to stop the carcinogenesis process in individuals with prior adenomatous polyps.

High-Fiber and High-Fruit and -Vegetable Trials of Polyp Recurrence

In the early 1990s, several cancer prevention trials tested the effect of a low-fat and high-fiber intervention to reduce adenomatous polyp recurrence (Table 2) (6, 11, 49, 58, 77). In each trial, individuals had undergone a complete colonoscopy and the removal of at least one adenomatous polyp. Eligibility criteria differed by trial according to polyp size, number, and time interval from polypectomy to enrollment. The intervention plan varied as well, from a wheat bran or fiber supplement, to combinations of low-fat diet plus wheat bran supplements, to an overall high-fiber, high-fruit and -vegetable, low-fat regimen. Actual percent of calories from fat on the intervention ranged from 20% to 25%, in contrast with the usual dietary regimen of 33% to 37%. The approach to fat reduction ranged from the removal of butter and/or visible fat from meat to the use of low-fat dairy products through the implementation of major changes in categories of food intake. In addition, several trials included a supplement of a micronutrient, such as 20 mg/d of β-carotene or a placebo (49). Given the randomization schema, as many as eight distinct combinations (including the placebo + usual diet group) of the trial might be tested in a factorial design. The length of the trials ranged from one to four years and assessment of outcome was blind, i.e., polyps were detected by colonoscopists who did not know of the patient's group status. The randomized trial design was followed according to strict guidelines established at the beginning of the studies, including random assessment of dietary compliance by dietitians (49), and annual completion of (previously validated) dietary

TABLE 2 Colorectal neoplasia prevention trials evaluating dietary fat, fiber, and fruit and vegetables

Reference	Sample size[a]	Design/cohort	Intervention	Primary results
DeCosse et al. (14)	58	DBRCT in FAP patients	Wheat bran fiber (2.2 g/d) + placebo versus wheat bran fiber (2.2 g/d) + vitamin C (4 g/d) + vitamin E (400 mg/d) versus wheat bran fiber (22.5 g/d) + vitamins C + E × 48 mo	Rectal adenoma number—nonsignificant reduction with high-dose fiber + vitamins
McKeown-Eyssen et al. (58)	201	Partially blinded RCT in patients with prior adenoma	Low fat (50 g/d or 20% of total calories) + fiber (≥50 g/d) versus customary diet × 24 mo (avg.)	Adenoma incidence/recurrence—no effect
MacLennan et al. (49)	424	Factorial, partially DBRCT in patients with prior adenoma	Low-fat intake (<25% of total calories) versus wheat bran (25 g/d) versus beta-carotene (20 mg/d) –7-arm factorial trial × 24–48 mo	Adenoma incidence/recurrence—no overall effect; reduction in large (≥1 cm) adenomas with combination of wheat bran + low-fat diet*
Alberts et al. (6, 17, 55)	1429	DBRCT in patients age 40–80 with prior adenoma (≥3 mm)	Wheat bran fiber 2 g/d versus 13.5 g/d × 36 mo	Adenoma number—1% reduction (NS); persons with adenomas—12% reduction (NS)
Schatzkin et al. (77)	2079	Partially blinded RCT in patients age ≥35 with prior adenoma	Low fat (</= 20% of total calories), fiber (18 g/1000 kcal), fruits and vegetables (5–8 servings/d) versus typical U.S. diet × 48 mo	Adenoma incidence/recurrence—no effect (RR = 1.00)
Faivre et al. (11, 18)	655	DBRCT in patients age 35–75 with prior adenoma	Fiber 3.8 g/d (ispaghula husk) versus calcium 2 g/d versus placebo	Adenoma incidence/recurrence—34% reduction with calcium (NS); 67% increase* with fiber
Women's Health Initiative (96a)	45,000–48,000	Complex factorial, 3 × 2 × 2 factorial DBRCT in postmenopausal women age 50–79	Low-fat diet versus calcium + vitamin D versus hormone replacement therapy × 9 yr	Colorectal cancer incidence—ongoing

*Statistically significant result ($P < 0.05$).

[a]Number randomized.

Abbreviations: DBRCT, double blind, randomized controlled trial; FAP, familial adenomatous polyposis; NS, nonsignificant; RCT, randomized controlled trial; SBRCT, single blind, randomized controlled trial.

food frequency questionnaires and multiple-day food records in randomly selected subcohorts of the Polyp Prevention Trial (PPT) participants (37). In addition, blood specimens were collected in the fasting state for measurement of micronutrient levels to assess biomarkers of dietary change (49). Nonetheless, the primary outcomes, notably the rate of recurrence in the intervention and control/usual dietary regimen groups, did not differ at the end of each trial. Therefore, there was no significant prevention of adenomatous polyp recurrence as a result of dietary intervention.

Several possible explanations were considered to explain why recurrence was not prevented or lower in those randomized to an intervention scheme compared to those on the placebo/usual diet, including the use of (*a*) the wrong endpoint, (*b*) an inadequate trial length, (*c*) the wrong timing in the life course for such a trial, (*d*) an insufficient sample size, and (*e*) an intervention that had been based on results of case-control, not prospectively collected cohort, research.

THE WRONG ENDPOINT By the time the trials were completed, it was recognized that $<10\%$ of adenomas developed into CRC, and not all CRC developed through the adenoma pathway. New mechanisms for CRC, such as microsatellite instability, were identified (80). In several trials, secondary outcomes such as the rates of large adenomas (≥ 10 mm) that have a greater malignant potential than small adenomas were reduced by an intervention, such as a low-fat diet plus wheat bran supplement (49), and the overall dietary intervention (high fiber, high fruit and vegetable, low fat) in the study (77). However, the sample sizes in the studies were too small to indicate whether the observed effects on this secondary endpoint are significant without further study.

INADEQUATE TRIAL LENGTH OR POPULATION The trial participants were adults with a history of at least one polyp prior to enrollment and therefore already at increased risk of developing another polyp. While they were at high risk of a recurrence, their mean age was typically in the sixties, with the potential that their colonic mucosa was less amenable to modulations (molecular, cellular, or tissue) from diet than a younger, healthier group without evidence of polyps. The duration of the intervention(s) had not been tested before the trials, either from observational research to define the time interval for optimal effect or from clinical nutrition research. Therefore, questions arose regarding the adequacy of the trial length; passive follow-up of trial participants frequently occurred postintervention (6, 77).

WRONG INTERVENTION Dietary change from a high-fat, low-fiber diet to a diet low in fat and high in fiber and/or fruits and vegetables provides leverage in the choice of food substitutes and therefore potentially more opportunity for long-term compliance. The downside of this broad application of dietary change is the difficulty in identifying which phytochemicals are consumed, the frequency and amount of intake, and whether food preparation and processing enhances or reduces absorption. For example, the length of time dark green leafy vegetables are cooked modifies folate concentrations and the amount of insoluble fiber, whereas reducing

water content from tomatoes progressively increases lycopene concentrations from raw tomato to sauce to paste.

Fruit and vegetables contain more than 25,000 recognized phytochemicals (13a). The search for the constituents in plants that provide protection involves identifying the major sources of variation. For example, seasonality and soil content can alter the concentration of phytochemicals, while inter- and intraindividual variability in intake of phytochemicals modify concentrations in human sera (11a, 51). Phytochemical databases to estimate intakes are limited to a few components, such as carotenoids (51). Thus, statistical analyses of the independent and combined effects of dietary constituents on cancer endpoints are difficult to determine. Dietary instruments used to assess intake have limitations from incomplete responses attributed to instrument length, and the number of days that can be reported; the respondent's accuracy in estimating portion size and frequency, which varies by the participant's education level and age. All of the above factors lead to measurement error (96). In the trials providing high-fiber supplements, adverse side effects were rarely mentioned; therefore, attrition from the inability to continue on the high-fiber regimen was a potential factor. In some trials, some patients refused to have the follow-up colonoscopy, thereby leading to selective subcohorts with endpoint ascertainment (58).

PRIOR EVIDENCE Reviews of earlier research revealed a consistent inverse association between fiber intake and CRC in case-control studies, whereas large population-based, prospective cohort studies such as the Nurses' Health and Health Professional Studies (20, 61) did not demonstrate such an association. Questions arose whether biases inherent to the case-control design led to spurious associations. For example, dietary reporting by patients *after* colon cancer diagnosis concomitant with telescoping of exposure assessment might bias the estimate of the effect of dietary fiber intake on cancer risk. Specifically, patients might report dietary intake during the period of appearance of symptoms and illness rather than prior to this period, which could lead to an effect when indeed it did not exist. Finally, exposures other than fiber intake, such as tobacco use, more consistently appeared as modifiable risk factors for adenomatous polyps (but not for CRC) than did one macronutrient in food (40).

AN UPDATE Two articles published in the *Lancet* in 2003 present data from observational, prospective, epidemiologic studies that suggest a significant inverse association between dietary fiber intake and risk of polyps (70) or CRC (10). Both studies had a larger and more varied range in dietary fiber intake (i.e., from 12–36 g/d) than in earlier cohort studies (20, 61). The major protective dietary source was fiber from grains, cereals, and fruit (70), and no food source of fiber was significantly more protective than others in the European Prospective Investigation into Cancer and Nutrition (EPIC) study (10). Interestingly, the use of fiber supplements was not associated with reduced risk. The adenomatous polyp study

(70) was based on sigmoidoscopy rather than colonoscopy for detection of polyps, which reduced the area of the colon that was screened and therefore the potential to identify all polyps. A major strength of both studies (10, 70) was that participants reported intake potentially reflective of chronic, long-term habits. Identifying the duration and phases of the life cycle during which the intake occurred would be a major contribution to further elucidating the timing of mechanisms of action in the natural history of polyps and CRC. Also, the two *Lancet* articles revealed a greater range in dietary intakes, presenting the possibility that previous dietary intervention trials did not use the optimal range in intake for reduction in polyp recurrence. Further analysis of the trials to explore the subset of individuals who met or went beyond the dietary goals of the trials might elucidate whether the duration of the trial was adequate to confer reduction in risk of polyp recurrence. Thus, dietary changes in fat, fiber, and fruits and vegetables did not confer protection from subsequent recurrence in the intervention trials, but new evidence indicates that a greater range in dietary intake might have an appreciable effect on polyp recurrence and CRC.

CASE SERIES III: CHEMOPREVENTION STUDIES OF RETINOIDS AND CANCERS OF THE UPPER AERODIGESTIVE TRACT

One of the first definitive proofs of principle for chemoprevention came from the translational studies of Ki Hong and colleagues, who used retinoids and other agents to suppress upper aerodigestive tract cancers. Clinical, epidemiological, and animal studies in the 1970s and 1980s suggested that vitamin A could positively influence epithelial cell differentiation, and thus retinoids may be effective agents for preventing epithelial cancers in the upper aerodigestive tract (34).

Chemoprevention of Upper Aerodigestive Tract Cancers by Retinoids

Hong et al. (27) established that high-dose 13-cis retinoic acid (13-CRA) is more effective than placebo in reversing oral premalignant lesions (OPLs), and later showed that low-dose 13-CRA is more effective than β-carotene and less toxic than high-dose 13-CRA (44, 69). These studies demonstrate that retinoids can indeed be used to reverse OPLs, as suggested by several reports in animal models (3, 87, 91). Further explorations of less toxic and more effective agents and regimens were accomplished by incorporating multidisciplinary studies of biomarkers into their trials and by the development of statistical methodologies for analyzing multiple biomarkers for the prediction of cancer development in patients with OPLs (39) (Table 3). Studies by Hong and colleagues (39, 89) demonstrate the importance

TABLE 3 Oral and aerodigestive cancer prevention trials evaluating retinoids

Reference	Sample size	Design/cohort	Intervention[c]	Primary results
Papadimitrakopoulou et al. (68)	36	Prospective nonrandom trial	Oral isotretinoin (100 mg/m2/d), oral alpha-tocopherol (1200 IU/d), and subcutaneous interferon alfa (3 megaunits per square meter twice weekly) for 12 mo	A striking difference in response was observed in favor of laryngeal lesions [9/19 (47%) complete response rate at 6 mo and 7/14 (50%) at 12 mo versus 1/11 (9%) and 0/7 (0%), respectively, for oral lesions]
Papadimitrakopoulou et al. (69)	59	RCT[a]	30 mg/d β-carotene, or 0.5 mg/kg/d isotretinoin for 12 mo	Isotretinoin 8% reduction β-carotene 55% reduction in oral premalignant lesions
Lippman et al. (46)	40	RCT	1.5 mg/kg/d isotretinoin or 13-*cis*-retinoic acid for 3 mo	Inverse relation of levels of accumulated p53 protein and response to isotretinoin in oral premalignancy
Lippman et al. (44)	70	Phase I; RCT in Phase II	1.5 mg/kg/d isotretinoin for 3 mo; pts w/ stable lesions given 1.5 mg/kg/d isotretinoin (n = 26) or 30 mg/d β-carotene (n = 33), for 9 mo	55% response in Phase I of 59 in the RCT, 92% versus 45% response in isotretinoin versus β-carotene arms
Hong et al. (28)	103	RCT	Oral isotretinoin (100 mg/m2/d) or placebo for 12 mo	No significant difference between groups in the number of local, regional, or distance recurrences of the primary cancers. However, the isotretinoin group had 4% versus 24% for the placebo group with second primary tumors
Hong et al. (27)	44	RCT	1-2 mg/kg/d 13 cis-retinoic acid or placebo for 6 months	A major decrease in lesion size in 67% (P = 0.0002); dysplasia reversal in 54%. Relapse occurred in 56%

[a]RCT, randomized controlled trial.

of conducting parallel basic and translational studies. They observed that the synthetic retinoid fenretinide induces apoptosis through retinoic acid receptor–independent mechanisms and induces cell death in cell lines resistant to all-trans retinoic acid, 13-CRA, 9-CRA, and other nuclear receptor–dependent retinoids (89). These findings have led to an ongoing trial of fenretinide in patients with retinoid-resistant OPLs and to the characterization of several novel retinoids with even more potent apoptosis-inducing effects than fenretinide (88). Further studies are needed to determine whether these agents reduce cancer incidence and mortality after treatment is completed.

Many groups studying chemoprevention of OPLs are currently moving away from retinoids and toward less-toxic agents, including bioactive food components such as vitamin E and green tea polyphenols, or pharmacologic agents that target specific pathways, such as inhibitors of farnesyl transferase, cyclooxygenase-2, or the epidermal growth factor receptor. However, these initial studies (39, 89) of retinoids were critical to establish the feasibility of developing a translational chemoprevention strategy.

These translational research findings (39) also led to new combination approaches to preventing OPLs. Retinoid resistance was shown to be associated with higher levels of genetic instability and mutant p53 expression (46). Combination regimens of 13-CRA, α-tocopherol, and interferon-α have been effective in reversing laryngeal premalignant lesions, but not OPLs (68), probably because some, but not all, of the p53-mutated OPL clones can be eliminated. These findings suggest that some genotypically altered clones can regrow and manifest as phenotypic lesions after treatment is discontinued (52, 78).

The Hong group (69) has also established that second primary tumors (SPTs), the leading cause of cancer-related death among individuals cured of an initial primary head and neck tumor, can be prevented by retinoid treatment (13-CRA). Patients definitively treated for an initial head and neck cancer showed a marked decrease in SPTs in response to a high-dose 13-CRA regimen for one year (28); however, significant side effects were associated with the high-dose 13-CRA treatment. Also, the effectiveness of the retinoid treatment diminished over time; in fact, the SPT rate by three years after cessation of 13-CRA treatment was the same as the placebo group (9). A follow-up study of the effect of low-dose 13-CRA for three years in preventing SPTs is nearing completion (33). Again, linking laboratory studies with these clinical trials has proven beneficial. For example, Hong, in collaboration with Margaret Spitz and colleagues, showed that susceptibility of peripheral blood lymphocytes to chromosomal breaks induced by the mutagens bleomycin or benzo[a]pyrene diol epoxide is an independent risk factor for head and neck cancer (82, 97). The mutagen-sensitive phenotype has also been shown to be significant predictor of SPT risk (83). The Hong group has illustrated the model of the multidisciplinary approach to cancer prevention that takes advantage of conducting basic research on biomarkers and clinical research on pharmacokinetics of the agent in the prevention of OPL and SPT.

CASE SERIES IV: CALCIUM SUPPLEMENTATION AND COLON POLYP RECURRENCE

The epidemiologic data on colon cancer incidence rates across the world as well as the increase in rates among recent Japanese migrants to Hawaii provided supporting evidence to undertake trials of diets high in fiber and fruits and vegetables as well as trials of calcium supplementation and adenomatous polyp recurrence (25). In addition, separate epidemiologic observations consistently demonstrated an inverse relation between dietary intake of calcium and incidence of colon cancer, as summarized by Sorenson (81). Animal research led by M. Lipkin and colleagues was the forerunner to the approach of identifying colon cell proliferation kinetics in mouse models and human subjects (43). This team moved back and forth between the two biosystems to develop the first multistage model of colonic tumor development in 1974 (42). Newmark & Lipkin have also demonstrated that calcium supplementation reduced cell proliferation in the mouse and human subjects at high risk of colon cancer (64).

Calcium Supplementation Reduces Polyp Recurrence

Amid the backdrop of epidemiological, clinical, and animal research, three trials of polyp recurrence were conducted in which calcium supplementation was administered with or without micronutrient supplements (Table 4) (8, 11, 23). The endpoint of polyp recurrence was recognized as a late intermediate biomarker of risk (8, 11, 23). Calcium carbonate was the form of supplement administered in doses ranging from 1.6–3 g/d; participants had a follow-up colonoscopy at years one and three of the trial, similar to the design of the fiber, fruit, and vegetable trials. Participants in the calcium supplementation arm of two of the three trials experienced a reduction in recurrence of 15% to 34% (8, 11), but no significant difference appeared in the rates of polyp recurrence in the third trial (23). Secondary analysis of the data in the third trial (23) revealed a reduction in recurrence among patients <65 years old and patients with only one adenoma at baseline. Several ongoing trials, such as the Women's Health Initiative, include a calcium supplementation arm (89).

Lipkin and colleagues recently completed a series of animal model experiments demonstrating the carcinogenic role of diet alone by de novo development of colon tumors in rodents administered a Western-style diet that was high in fat, low in fiber, and low in calcium and other micronutrients associated with cancer prevention (65). This same team reported the protective effects of dietary calcium add-back to reduce risk of colon tumors in mice that had been fed the Western-style diet compared with rodents who remained on the diet alone and others who remained on the control diet (43). This team's research, the forerunner of many of the animal-human cancer prevention trials, has moved from the arena of chemoprevention to demonstrating carcinogenic capacity of diet alone. The challenges rest in how well the Western-style rodent diet is mirrored in the complex arrays of current

TABLE 4 Colorectal neoplasia prevention trials evaluating calcium

Reference	Sample size[a]	Design/cohort	Intervention[b]	Primary results[c]
Hofstad et al. (23, 24)	116	RCT in patients with current adenomas (those <1 cm were retained)[d]	1.6 g/d + beta-carotene 15 mg + vitamin C 150 mg + vitamin E 75 mg + selenium 101 mcg/d × 36 mo	Growth of small adenomas—no effect; adenoma incidence/recurrence—increased*; fecal bile acids—no effect
Baron et al. (8)	930	RCT in patients with prior adenoma	3.0 g/d × 48 mo	Patients with adenoma recurrence—19% reduction*; adenoma number—24% reduction*
Faivre et al. (11, 18)	655	RCT in patients with prior adenoma, age 35–75	2.0 g/d (versus 3.8 g/d ispaghula husk) versus placebo × 36 mo	Adenoma incidence/recurrence—34% reduction (NS)
Women's Health Initiative (96a)	45,000–48,000	Complex factorial, RCT in postmenopausal women age 50–79	Calcium + vitamin D; hormone replacement therapy; low-fat diet × approximately 9 yr	Colorectal cancer incidence (among many others)—ongoing

[a]Number randomized

[b]Calcium carbonate unless noted otherwise

[c]Proliferation assessed via random biopsies of normal appearing mucosa

[d]RCT, randomized controlled trial; NS, nonsignificant

*Statistically significant result ($P < 0.05$)

Western-style human diets and the identification of the windows of human development that are sensitive to their reported dietary modulations.

WHERE DO WE GO FROM HERE? CONSIDERATIONS AND POTENTIAL ACHIEVABLE OBJECTIVES BASED ON LESSONS LEARNED FROM EARLIER TRIALS

In this section, the scope of cancer prevention research is described and the lessons learned from the case studies of nutrition trials of cancer prevention are discussed. We highlight a multidisciplinary approach to nutrition and cancer prevention research that integrates the research expertise and insight from basic, clinical, population, and translational science (Figure 2).

What is Cancer Prevention Research?

Historically, cancer prevention research has focused on reduction in incidence or mortality from cancer. The definition has broadened over the past decades to include any intervention that delays the process of carcinogenesis (85). Cancer is a multistage process; therefore any factor or exposure that impedes, blocks, or delays one or more steps without conveying pathogenesis of another disease process would be classified as a prevention approach today. We have moved from the arena of reducing secondary cancers (27) and recurrence of adenomatous polyps (6, 58, 77) to molecular targets and pathways, such as caloric restriction to modulate insulin-like growth factor-1 (32) and nonsteroidal anti-inflammatory drugs or dietary bioactive anti-inflammatory components to modulate prostaglandin biosynthesis (92). Multiple stages of the life cycle might provide age or developmental-stage sensitive windows of opportunity for different (combinations of) preventive approaches or intervention modules.

What Have We Learned from the Successes and Failures Described in Earlier Trials?

As is frequently the case, the reasons for a failure are explored more than the nature of a success. Insufficient groundwork before beginning a randomized clinical trial of nutrition interventions in cancer prevention may be one of the main lessons to be learned. For example, pharmacodynamic and pharmacokinetic studies to identify the optimal dose and duration were not completed before the β-carotene trials; thus, important data were not available that demonstrated large interindividual variability in response to a supplement (16). Relevant animal experiments had not been conducted in terms of molecular targets and agents. Lifestyles and behaviors such as smoking or drinking alcohol put participants in several β-carotene trials at high risk for a specific cancer. However, phase I and II studies were not conducted in these high-risk groups using pharmacologic doses of naturally

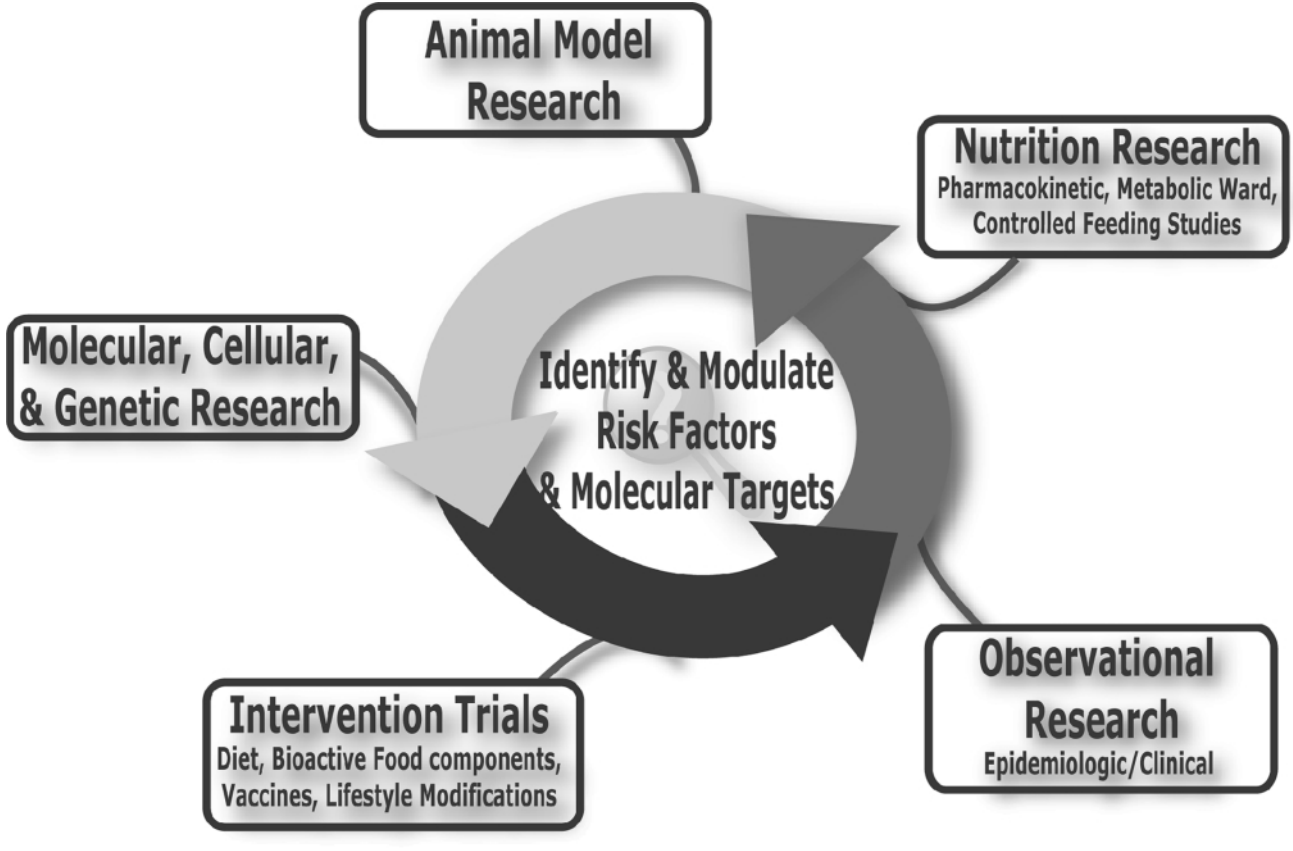

Figure 2 The wheel of multidisciplinary research in nutrition and cancer prevention.

occurring substances to assess toxicity before the trials began. Likewise, preclinical experiments of supplementation administered to animals exposed (or not exposed) to smoking chambers (and alcohol intake) might have detected adverse outcomes prior to the human interventions (66). In contrast, Hong and colleagues examined gender-specific effects of 13-CRA and reported a significant decline in mean serum retinol levels in women, but not men (45). This team also tested the combination of alpha-tocopherol and 13-CRA to reduce toxicity from the retinoid, and indeed, the combination of agents had lower initial rates of toxicity than the single retinoid agent (15). Recognizing that toxicity may far exceed the benefit of synthetic agents, Hong et al. have moved into the arena of bioactive food components as potential replacements for retinoids.

In addition to the problem of insufficient groundwork before trials were conducted, dissemination and synthesis of findings from clinical and epidemiological research and from animal experiments might not have occurred. Cross-fertilization between human and animal research could potentially identify gaps in research areas ranging from the development of food composition databases to the demonstration of the optimal duration of and compliance with an intervention. Indeed, individual agents were the targets for interventions in the β-carotene trials, when multiple foods (fruit and vegetables) were associated with reduction in cancer risk or the food pattern might have been an indication of a healthy lifestyle of the respondent. Moreover, when overall dietary change was the mode of intervention as in the PPT (37), the advantage of having the participant select foods to modify in the diet may have been offset by the disadvantage of inadequate food composition databases to assess the effect of individual or combinations of bioactive food components on recurrence. Thus, the state of the art of dietary assessment to evaluate participants' responses was not ready for the test of the trial, even under conditions where the tools of the trade, notably food frequency questionnaires and food records, had been validated prior to the trial and participants had been extensively trained in reporting dietary intake (37). Finally, the majority of epidemiologic findings supporting an intervention arose from case-control studies, with inherent potential for biased reporting of diet following diagnosis, while cohort studies had insufficient range of dietary intake to assess dose and duration for prevention. The recent EPIC study of CRC (10) demonstrates a breadth of dietary intake in relation to cancer risk, which ameliorates this issue initially presented in the work of Mertz (60).

The successful trials have demonstrated proof of the principal that nutritional interventions can reduce the risk of carcinogenesis from precursor lesions to second primary tumors. One aspect characteristic of success is the integration of animal and clinical research into translational science, i.e., recognizing the importance of linking molecular, animal, and clinical studies for a tremendously powerful approach to cancer prevention (26, 29, 43). Three aspects of this integrated approach include (*a*) testing the efficacy of the particular agent and identifying potential targets for other agents or combinations of agents; (*b*) having the tools available to test the efficacy of an agent at the same time as examining whether the agent confers protection from cancer and also increases risk of another disease, thereby

addressing the agent-target fit much like the person-environment fit of earlier epochs; and (*c*) having the ability to accelerate the pace at which interventions are moved from the bench to bedside. Given the complexity of pathways to chronic disease, agents such as calcium have the capacity to enhance prevention of multiple endpoints, including recurrence of polyps and loss of bone density, both of which are important for quality of life. Animal research has come closer to the human diet and has demonstrated the efficacy of a bioactive component, such as an olive oil, fruit, and vegetable mixture, as well as the efficacy of calcium (50, 65).

Reaching for a Multidisciplinary Approach for Nutrition and Cancer Prevention

Within the genre of prevention as it is embraced today, a multidisciplinary team is required to effectively develop and conduct future prevention trials (Figure 2). Members of this team include epidemiologists and biostatisticians with expertise in clinical trial design and analysis of gene-environment interactions; nutrition experts in pharmacokinetics, dietary interventions, animal experiments, and dietetics; molecular and cell biologists; and behavioral scientists. What can we gain from experts in these fields? Data from the pooled observational epidemiologic cohort studies in humans have the statistical power to detect gene-environment interactions that might influence the carcinogenesis process by conferring protection or enhancing risk. New statistical approaches are being developed to estimate the magnitude of such an interaction on specific molecular pathways (13). Characteristics of participants in the pooling project can be compared with those of volunteers in past intervention trials in an effort to define a new profile of the healthy volunteer for future prevention trials. By way of illustration, one observes that the overweight/obese has filtered through prevention trials over time as evidenced by an average BMI of 27 (i.e., within the category of overweight, not normal weight) for participants in the Women's Health Initiative (59) and the PPT (77), and even as far back as the Multiple Risk Factor Intervention Trial (36). Thus with the overweight being represented in prevention trials for decades, prevention research has to take a good look at the eligibility criteria for volunteers and whether those who meet the criteria are normal, healthy individuals who will be able to comply with the intervention goals.

Observational epidemiology can provide clues to developmental windows of susceptibility to disease and can identify targets for nutritional interventions (M.S. Linet, M.R. Forman, L.M. Anderson, M.A. Smith, & L. Ries, submitted). Findings from epidemiology studies can be tested in animal models to determine whether the nutritional intervention will hit the molecular target, can modulate the developmental window, or have long-term preventive or toxic effects.

ANIMAL MODELS STUDIES Studies in animal models can test interventions across the full spectrum of the carcinogenesis process and identify their impact (both positive and negative) on the entire biosystem. Relevant animal models can target

specific pathways, and when necessary there are approaches to humanize the rodent; as for example, cytochrome P450 isoforms function analogously in animals as they do in humans (F. Gonzales, personal communication). Animal experimentation is more amenable to modulating the phenotype by body mass or other characteristics (e.g., physical activity) associated with risk of human disease, and to contrasting varying doses of the intervention to assess effect. We need to recognize the limitations, however, of the rodent and other animal models for application to human prevention research and, when necessary, develop new models that are more appropriate. For example, β-carotene supplementation is best examined in the nonhuman primate rather than in the rodent because of differences in adipose tissue absorption. Certain rodent models activate or inhibit specific stages of carcinogenesis or molecular pathways to cancer, which is an attractive feature. By accelerating the carcinogenesis process we can look more rapidly at the chemopreventive effects of an agent. However, such rapid models may be too aggressive and may lack the subtlety, long latency, and complexity of the human process of cancer.

MOLECULAR TARGETED RESEARCH Rapid advances in several fields, including cancer therapy, have been achieved through molecular targeted research. However, the fields of nutrition and cancer prevention have not yet sufficiently capitalized on recent advances in molecular carcinogenesis or on the increasing availability of new technologies and approaches, such as genomics, proteomics, metabolomics, imaging, and genetically engineered mouse models. Evidence is accumulating that a large number of bioactive food components can exert effects on the human genome, either directly or indirectly, to modulate gene expression (95). Furthermore, many diet-related genes are involved in the carcinogenesis process (31). It has also become clear that the degree to which diet influences health of the biosystem often depends on genetic constitution and metabolic state. Future progress in nutrition and cancer prevention will be facilitated by the integration of molecular targeted approaches into epidemiologic, clinical nutrition, and animal model studies. This will accelerate the pace at which we fill key gaps in this field, such as elucidating mechanisms underlying effective nutritional modulators of carcinogenesis and assessing the specificity between nutrients and their targets, identifying important interactions between dietary factors, and discovering and validating biomarkers for nutrition and cancer prevention studies (63).

CLINICAL NUTRITION RESEARCH: THE POTENTIAL GLUE TO CONNECT ANIMAL EXPERIMENTS TO HUMAN TRIALS AND REPLACE TRIALS OF NUTRITION INTERVENTIONS IN CANCER PREVENTION The scope of clinical nutrition encompasses acute phase kinetic research to controlled feeding studies of moderate-sized groups and research in the free-living state. The exposure is commonly limited to either a specific dose or, for example, a constant daily amount of alcohol intake over a specified period that does not mimic conditions in the free-living state. Yet, clinical nutrition research has the opportunity to broaden exposures, their dose, and duration, as well

as to modulate several exposures at the same time. For example, the availability and use of exercise treadmills in the clinical nutrition unit provide the opportunity to assess the effect of energy expenditure from lifestyle interventions in various high-risk phenotypes for cancer and other chronic diseases.

Controlled feeding studies are traditionally conducted under isocaloric conditions, thereby reducing the effects of modulations in weight gain and loss on the study endpoint. This approach has benefits and detriments for development of future nutrition prevention trials. The benefits are the ability to test a pharmacologic agent such as vitamin E supplementation without the potential confounding of changes in body fat mass. The detriment may be the inability to document how an intervention, such as a change in the overall dietary plan from a traditional Western high-fat and low-fiber diet to low-fat and fiber-rich diet, reduces weight and thereby alters hormonal profiles related to risk of cancer.

Human intervention studies can test the efficacy of combined agents, vaccines, or preventive behaviors such as weight reduction and smoking cessation on molecular targets, biomarkers of disease, or reduction of comorbidity in cancer patients. New molecular technologies such as proteomics and genomics offer the potential to discover nutrient-gene interactions and biomarkers of disease. Challenges to future research include identification of the optimal duration and dose of the intervention(s) for effective prevention. The diversity of clinical nutrition research will depend on the creative energies of the multidisciplinary team, the willingness to move into at-risk populations by virtue of family history or current health status, and the ability to utilize new technologies, agents, and animal models to inform the research process. Indeed, investigators may never reach accord on an optimal diet or lifestyle intervention for cancer prevention to test in a large randomized, clinical trial; rather, the clinical nutrition research engine could, in conjunction with the animal model system, be the driving force toward new approaches to nutritional interventions in cancer prevention research.

ACHIEVABLE OBJECTIVES

Various avenues toward achievable objectives arise by extrapolating lessons learned from earlier trials, and by examining the attributes of the multidisciplinary research wheel (Figure 2). Specifically, research in nutritional interventions for cancer prevention should consider the following:

- Ascertainment of multiple endpoints, not just cancer
- Examination of the complexity of common molecular targets that confer protection or risk of a chronic condition
- Interpretation of research from animal and human studies with an understanding of the limitations of the study design, the animal models, and the characteristics of the human population studied
- Identification of potential windows of susceptibility for interventions that

might be associated with different doses and durations of interventions as well as different high-risk subgroups of the population

- Combinations of a range of prescriptives from whole-food dietary plans to bioactive food components, pharmacologic agents, vaccines, and/or lifestyle modifications
- Assessment of variability (intra- and interindividual) in response to the preventive measure(s) before conduct of the large-scale research
- Specification of the range in population subgroups among whom the prevention would be most effective
- Evaluation of shared (or not shared) factors related to intermediate markers/precursor lesions and to cancer endpoint
- Recognition of multidisciplinary contributions from human research that inform animal experiments, and vice versa
- A balanced perspective on prevention and aging as a biologically natural phenomenon.

In summary, randomized clinical trials are the recognized gold standard to evaluate causality, and many trials of nutrition and cancer prevention have provided valuable lessons. The β-carotene lung cancer trials and the retinoid upper aerodigestive tract trials had surprising and recognized toxicities, respectively. The fiber and fruit and vegetable trials of polyp recurrence demonstrated the limitations of dietary change on late intermediate markers of cancer. Through the interface of animal and human explorations, calcium supplementation arose as a successful intervention for polyp recurrence and colon neoplasia with benefits for the overall biosystem. Future progress in nutrition and cancer prevention will depend on the confluence of energies and expertise from a multidisciplinary team. Informed observers of the evidence have the challenge to modulate diet and other lifestyle factors associated with carcinogenesis and to assess the preventive capacities of other modalities on tumorigenesis. Clinical nutrition strategies with validated surrogate endpoints in humans and molecular-targeted interventions in tandem with strong mechanistic studies in animal model systems may be the modus operandi in lieu of the randomized clinical trial.

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Annual Review of Nutrition
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CONTENTS