

Plant Foods versus Compounds in Carcinogenesis; Observational versus Experimental Human Studies

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Received for publication: November 4, 2002

Abstract: The protective role of plant foods and its constituents in cancer prevention is under renewed debate since the results of recent observational studies on colorectal cancer as well as large-scale human experimental studies on colorectal adenoma recurrence are disappointing. However, most short-term experimental human studies do show that plant foods favourably modulate potential cancer-preventive mechanisms. Which methodological pitfalls may explain the inconsistencies within and between different study designs? What are the advantages and limitations of the different study approaches?

Observational studies do have the advantage to study the population at large with ultimate disease as the study endpoint. These studies are limited by the difficulty to estimate intake of individual compounds by questionnaires and the lack of biological markers of relevant exposure. Controlled experimental short-term studies in humans rely on biological markers of disease as intermediate endpoints. Relatively low sensitivity and specificity of these markers may complicate extrapolation of results. In the case of long-term and large-scale human intervention studies with disease endpoints, issues such as time, dose and duration of intervention, compliance and choice of the study population influence the interpretation of results.

An integrated approach combining designs, and implementing new techniques to identify biomarkers, may clarify the role of plant foods in carcinogenesis.

Key words: Vegetables, fruits, bioactive compounds, epidemiology, intervention study

Introduction

A diet high in foods of plant origin may favourably influence health and prevent chronic diseases, such as cancer. Experimental animal studies and *in vitro* investigations support this protective effect of plant foods, although it is still speculative which specific constituents of plant foods are responsible. Observational and experimental studies in humans are also mainly supportive, but results of recent

large observational and intervention studies on colorectal cancer are disappointing. Several design issues may explain the inconsistent findings of observational and experimental human studies. This paper provides a brief overview of observational and experimental studies on plant foods, specific non-nutrients, and cancer. Individual studies are used as examples to discuss the advantages and the limitations of observational and intervention studies on plant foods.

Observational studies

Plant foods and cancer

Epidemiologic data support the apparent inverse association between vegetable and fruit consumption and cancer risk as demonstrated in a comprehensive review published in 1997, including more than 250 case-control and cohort studies [1]. About 70% of these studies show a decrease in cancer risk with higher intake of fruits; about 20% show no association, while 10% suggest an increased risk with relatively high fruit intake. For vegetables, 80% percent of the observational epidemiologic studies reviewed show a protective effect, about 5% of the studies did not show an association and about 15% of the studies supported an increase in risk with higher vegetable consumption. Relative risks (RR) or odds ratios (OR) ranged from 0.3 to 1.5. Despite variations across studies, it was concluded that convincing evidence for inverse risk associations were observed for vegetables and fruit intake for cancers of the mouth and pharynx, oesophagus, lung, stomach, colon and rectum [1].

For colorectal cancer, however, recent prospective cohort studies do not support a strong significant inverse association with vegetable and fruit consumption. The IOWA Women's Health Study among 40 000 post-menopausal women was among the first cohort studies which did not find a significant protective effect of either fruit and vegetable intake after 5 years of follow-up [2]. The combined data from the Nurses' Health Study and the Health Professionals Follow-up study with nearly 2 million person-years of follow-up, including 1100 incident cases of colon and rectal cancer did support these null-findings [3]. A difference in fruit and vegetable consumption of one additional serving per day was associated with a co-variate adjusted RR of 1.02 (95% Confidence Interval (CI) 0.98–1.05) for colon cancer. For rectal cancer, similar findings were observed [3]. Also, the Netherlands Cohort Study on Diet and Cancer among 120 000 Dutch subjects, including more than 1000 cases of colon and rectal cancer after 6 years of follow-up, does not support a statistically significant inverse association between total vegetables, fruits and colorectal cancer [4]. Only for brassica vegetables, which in the Netherlands will be mainly cauliflower and brussels sprouts, and for cooked leafy vegetables, which includes spinach and endive, decreased risk with higher consumption was observed [4]. The relation between Brassica vegetables and cancer has been reviewed by van Poppel and colleagues in 1999 [5]. They conclude on the basis of the results of 6 cohort and 74 case-control studies that high Brassica consumption is associated with a decreased risk of cancer, mainly lung, stomach and colorectal cancer.

Other plant foods, which may exert a protective effect on cancer, are whole grains. As compared to vegetables and fruits, and despite considerable attention on dietary fibre (see below), relatively few epidemiologic studies have specifically investigated the association between wholegrain cereals and cereal products and cancer risk [1]. Jacobs [6] reviewed more than 50 case-control studies on various gastrointestinal and hormone related tumours. Out of 45 studies without design or reporting flaws or low intakes, 43 studies showed an inverse association with high intake of whole grains. The pooled OR for high versus low whole-grain intake among the 45 studies was 0.66 (95% confidence interval = 0.60–0.72) [6]. Studies on dietary fibre from vegetables, fruits and whole grains show, however, inconsistent results (see below).

Tea is an important beverage of plant origin. World-wide per capita consumption of tea is with about 120 ml/day second to water only [7]. Epidemiologic studies regarding the association between black and green tea and cancer have been inconclusive, but point to the possibility of lowered risks of digestive tract cancer among tea drinkers [8, 9]. However, we recently found that in a Dutch cohort of elderly men, tea consumption was unrelated to incidence of epithelial cancers in general (RR 1.00, 95%CI= 0.95–1.04) [10], confirming data from three previous studies where tea consumption was found to be unrelated to total cancer mortality or incidence [11]. Studies on the relation between tea and lung cancer have generally been negative: five cohort studies and three case-control studies reported that there was no association between tea intake and lung cancer risk [8, 10, 12]. One cohort study showed an increased risk in lung cancer with a high intake of tea, which was ascribed to confounding because of the limited adjustment for smoking and the reported positive association between smoking and tea consumption [8]. Data for the two major other epithelial cancers, colon and kidney/bladder, generally show no association for tea intake and kidney/bladder cancer, and inconsistent data for colon cancer [8, 13]. Most literature does not support an inverse association between tea consumption and rectal cancer [e.g. 13]. A major drawback of all these studies, except for the cohort studies and the one case-control study that reported an inverse association, is that they were conducted in countries where black tea consumption is extremely low (Japan and Italy) and therefore unlikely to exert any physiological effects.

Non-nutritive compounds of plant origin

Many phytochemicals may be responsible for the potential protective effects of Brassica, whole grains, tea and other foods and beverages of plant origin. Table I provides an overview of the main non-nutrient constituents of plant foods.

Table 1: Plant foods and non-nutritive constituents potentially related to cancer prevention

Major source	Family of phytochemicals
Cruciferous vegetables	Indoles, glucosinolates, isothiocyanates
Allium vegetables and (e.g. garlic, onion), Cruciferous vegetables (e.g. broccoli and cabbage)	Organosulphur compounds
Soybeans	Isoflavones
Coffee, cereal bran, apples	Phenolic Acids
Tea, onions, apples, red wine	Flavonoids
Legumes	Saponins
Whole grains	Lignans
Vegetables and fruits (e.g. citrus fruits)	Terpenes

For instance, Brassica vegetables contain glucosinolates that are hydrolysed to indoles and isothiocyanates. A Chinese case-control study (including 233 female cases; 187 hospital-based female controls) on the association between dietary isothiocyanates and lung cancer was recently published [14]. Internal exposure to isothiocyanates was assessed by questionnaires and for some of the most important foods isothiocyanates were determined by HPLC. The study showed an inverse association with isothiocyanate intake (OR = 0.6, 95%CI = 0.4–0.95) for weekly intake of isothiocyanates (53.0 compared to >53.0 (mol), which was especially strong among smokers (OR = 0.3, 95%CI = 0.1–0.96) and among those who lack expression of the GSTM1 gene (OR = 0.6, 95%CI = 0.3–0.9). The GSTM1 gene codes for the enzyme GSTmu which is involved in the conjugation and excretion of isothiocyanates. No activity of this enzyme might mean that isothiocyanates are not easily excreted and may therefore exert their favourable effects for a longer time. Whether this is actually true, and whether this oversimplifies the actual underlying mechanism can not be evaluated by these types of studies.

Traditionally, the potential protective effect of high whole-grain consumption has been explained by dietary fibre. Although results from case-control studies on dietary fibre and colorectal cancer risk remain promising [15], recent prospective cohort studies [16, 17] as well as large-scale intervention trials (see below) do not support the dietary fibre hypothesis. As an alternative explanation, the beneficial effect of whole grain products might be related to the lignans present in whole grains. Lignans are constituents of plant cell walls, which explains the correlation between high fibre intake and lignans. Apart from whole grains, they also occur in seeds, tea and some vegetables [18]. The major plant lignans are secoisolarici-

ciresinol (SECO) and matairesinol (MAT), however, additional partly unknown lignans exist [19]. Lignans are converted to enterolactone (ENL) and enterodiol (END) by bacteria in the colon [20]. It was found that whole grain food intake elevates serum ENL [21]. ENL and END are generally present in human urine, plasma, and faeces, which provides opportunities for use as biological markers of exposure. A recently published case-control study on phytoestrogens and breast cancer did not rely on questionnaires but on measured metabolites of plant lignans in serum [22]. They observed a strong inverse association between ENL and breast cancer risk (RR more than 35 versus less than 6 nmol/L = 0.4, 95%CI = 0.2–0.8). In this Finnish study, levels of ENL were explained by rye bread and dietary fibre. However, these factors only explained a small part of ENL levels. The authors expect that ENL levels may also be explained by the composition of the intestinal microflora, genetic background, medication, alternative sources of lignans, etc. [23]. Epidemiological studies on the relation between plasma ENL and colorectal cancer have not yet been published. The intake of lignans is heterogeneous in western countries; the between persons coefficient of variation of urinary excretion of enterolactone was 50% in Dutch women [24]. This illustrates their potential relevance to diet-related variation in disease risk. However, data on lignan contents of foods are scarce and incomplete.

Flavonoids are major constituents of tea and may therefore be responsible for observed potential health effects of a high tea intake. Tea is a particularly rich source of two major subclasses of flavonoids, the flavonols and the catechins. We are aware of eight reports, all but one from prospective cohort studies, on the relation between intake of flavonols or catechins and cancer risk. Figure 1 provides an overview of the studies conducted this far on flavonols and cancer. Of these, three found no association between total flavonol intake and lung cancer [12, 25, 26] or cancer of the alimentary and respiratory tract [27], whereas two reports from the same study in Finland found a statistically significant reduction in lung cancer risk [28, 29]. In another Finnish study, the ATBC study, the intake of flavonols was inversely associated (RR = 0.6) with lung cancer risk in male smokers [30]. None of the studies found flavonol intake to be related to colorectal or stomach cancer. As yet, only two epidemiologic studies have reported on the association between catechin intake and cancer incidence. Among several cancers studied, catechin intake was inversely associated with rectal cancer risk only [10, 31].

In summary, observational studies on plant foods, plant constituents and cancer show inconsistent results. These inconsistencies may be related to genetic and lifestyle differences between the populations studied or to study design issues.

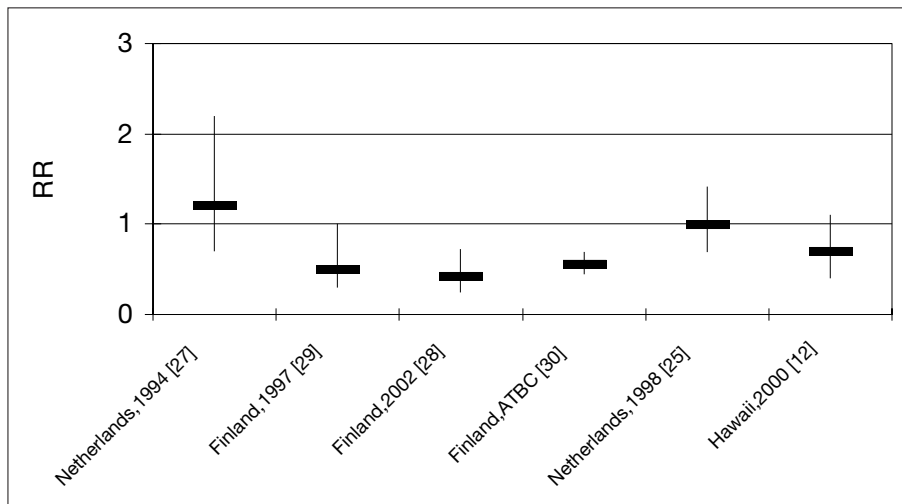


Figure 1: Flavonols and cancer: 5 prospective studies and 1 case-control study.

Advantages and limitations of observational studies

Observational epidemiologic studies are studies among the species of interest. Through observational studies we are able to study associations with disease inducing agents, which would be unethical in experimental human trials. Moreover, we are able to study the population at large and not only a specific high risk group. These population studies are dealing with physiological levels of intake, normal ranges of intake, which are nicely extended to preventive strategies. Also, the follow-up of prospective studies is more compatible with disease development than we could expect from experimental studies. A drawback is that observational studies show associations: causality may be deducted only by inference.

Several design issues need to be considered when interpreting epidemiologic data [32]. Case-control studies are in general more susceptible to various forms of biases as prospective cohort studies [33]. Selection bias may especially be important because of the absence of patients who do not survive long enough to get into the study. There is also the potential for bias as a consequence of possible differences in recall of diet between patients with disease and controls. However, results of cohort studies also need to be interpreted with caution. Although it is true that comparisons made within a cohort are relatively unbiased, the cohort itself may be quite unrepresentative of the general population, because of the specific population targeted for recruitment and the degree of subsequent self-selection into the cohort. This recruited population may differ from the general population in terms of the behaviour under study and in terms of the clustering of other causal and protective factors. Another common issue in cohort studies, may be the increasing time from measured exposure

to the onset of disease. To overcome this potential problem, some cohort studies (e.g. [16]) use an updated measurement of exposure.

In observational studies it is notoriously difficult to assess the exposure to the food or compound of interest. Most observational studies still mainly rely on questionnaires as far as intake levels are concerned. Databases of non-nutrients are scarce and incomplete. Not only is it hard to estimate intake in observational studies, they are also limited by the fact that internal exposure to bioactive compounds is difficult to evaluate. Bioavailability of compounds, tissue distribution, the influence of the microflora and the influence of genetic variants in all metabolising enzymes involved is difficult to take into account. Therefore, we are still in need of good, reliable, efficient biomarkers of long-term exposure to be used in large observational studies, such as the metabolites of plant lignans in the previously mentioned study on breast cancer [22].

Also, the range of intake in observational studies may be insufficient to assess an association. International studies, with a sufficient variation in intakes, such as the European Prospective Investigation into Cancer [34] may help to tackle that problem.

Even though most studies adequately adjust for confounding and modifying variables, residual confounding may still be present because of factors which are not, maybe could not be taken into account. Healthy behaviours tend to cluster. Smoking, the single most important risk factor for many cancers is associated with a higher intake of energy, alcohol, and fat, with a lower intake of fruits and vegetables, lower socio-economic status, and with physical inactivity [35, 36, 37]. Previous studies have shown that consumption of tea in the Netherlands [38] and in Japan [39], is associated with a healthy dietary pattern.

This creates difficulties in interpreting findings of observational studies because of residual confounding.

A related problem is that observational studies focusing on individual compounds may be hampered by high correlations between compounds. Each food contains a large number of different compounds, some known and quantified, some less well characterised, and some as yet unknown or not measurable. Many compounds tend to be present in the same foods or families of foods. For the intake of vitamin C, beta-carotene and fibre, correlations in several European populations were reported of the order of 0.40 to 0.70 [40]. It is often difficult in these studies to distinguish the effects of vitamins from those of isothiocyanates, lignans and other bio-active compounds. (See also [41]).

Experimental human studies

Short-term studies with intermediate endpoints

Small-scale, short-term experimental human studies focusing on intermediate endpoints are mainly conducted to evaluate the potential modes of action by which plant foods and its constituents may protect against common diseases. Table II shows potential effects for disease prevention which may be identified in human studies (adapted from [42]).

Antioxidant activity may be one of the most important cancer-related protective activities of plant foods. For instance, in a Dutch experimental study [43], 10 volunteers were given 300 grams of Brussels sprouts per day for three weeks. In urine, 8-oxodeoxyguanosine was declined in men as a result of the intervention.

Anti-carcinogenic activity of tea and its major flavonoid constituents has been demonstrated in many animal models and at different stages of the carcinogenic process [44]. Human evidence was scarce until recently. In the past few years the antioxidant capacity of tea has been evaluated in several short-term human trials. Re-

peated consumption of tea or tea extracts for one to four weeks has been shown to decrease a variety of indicators of oxidative stress [7]. A double-blind, placebo-controlled trial in 59 patients with oral precancerous mucosa lesions resulted in partial regression of the lesion in 38% of the patients treated with oral and topical administration of black and green tea mixture [45].

Another potential mechanism by which plant foods may favourably influence health is by modulation of phase II detoxification enzymes [42]. Many studies examined the influence of plant foods on the activity and expression of phase II detoxifying enzymes. For instance, increased GST activity was observed after consumption of Brussels sprouts and broccoli [46]. Increased glucuronidation was observed in feeding studies with Brussels sprouts, cabbage and watercress [47, 48]. Recently, a randomised cross-over feeding study including 43 participants consuming four different plant food diets (basal, Brassica, Allium and Apiaceous) for 7 days was conducted [49]. For Brassica, a significant increase in serum GST alpha activity was observed for those with the GSTM1 null genotype. Also, Brassica and Allium vegetables increased white blood cells GST-mu activity in women with the GSTM1 gene, which was not observed among men [49].

ENL and END show weak estrogenic and antioxidant activity [20]. They have been implied in inhibition of cell proliferation and induction of quinone reductase [50]. Experimental studies in human have not been published this far [51].

Long-term studies with disease endpoints

To evaluate whether these observed influences on antioxidant response and enzyme activities ultimately influence health, large-scale, long term human intervention studies with validated intermediate disease markers or ultimate disease endpoints are necessary. Up until now no large scale, population-based, long term intervention studies have been published on isolated non-nutrient bioactive compounds from plant foods. But there have been several large-scale studies looking at high fibre foods and polyp recurrence after three and four years. These include the well-designed US Polyp Prevention Trial [52], the Phoenix trial [53], and the ECP trial [54], none of them showing promising results (as depicted in Fig. 2). The Polyp Prevention Trial is a randomised, multicenter placebo-controlled, double-blinded intervention study. About 1000 people were counselled and got dietary advice to decrease their fat intake and increase fibre and fruits and vegetable consumption. The other 1000 participants remained on their usual diet. The intervention group increased their fibre intake by 75% and their fruits and vegetables intake by 66%. Men and women with previous colorectal ade-

Table II: Potential mechanisms for disease prevention identified in human studies (adapted from [42])

☐ Antioxidant activity
☐ Modulation of detoxification enzymes
☐ Stimulation of the immune system
☐ Decrease in platelet aggregation
☐ Modulate steroid hormone concentrations and hormone metabolism
☐ Blood pressure reduction
☐ Antibacterial and antiviral activity

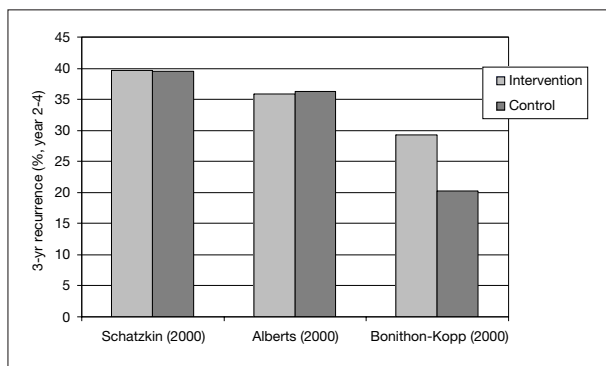


Figure 2: Vegetables, fruits and dietary fibre: large-scale intervention studies.

nomas were included in the study. After 4 years of follow-up (and excluding the first year of follow-up) no significant difference in adenoma recurrence was observed [52].

So, up until now no large intervention studies have been conducted supporting that vegetables, fruits, or fibre do have a strong effect on cancer. Although, these experimental studies would provide the strongest evidence for an effect of vegetables and fruits on disease risk, also these studies have their limitations.

Advantages and limitations of experimental studies

Obviously, controlled experimental studies are less influenced by some of the potential biases of observational studies, such as recall bias and residual confounding. However, some of the inconsistencies between experimental and observational studies may be attributable to the use of inappropriate effect biomarkers in experimental studies. The validity of experimental feeding studies using either whole-diet changes or supplements depends critically on biomarkers that serve as surrogate endpoints of disease outcome. The sensitivity and specificity of these markers may sometimes not be tested or may be disappointing. For example, many markers of oxidative damage used to study effects of antioxidants such as TBARS or strand break analysis alone, are now considered inappropriate for most applications [55].

Because of high cost, long duration, and ethical issues, interventions with clinical disease outcomes are often unfeasible. When feasible, however, these large-scale interventions also do not offer conclusive evidence that vegetables and fruits do not protect against colorectal cancer (see also [56]). Issues such as time and duration of intervention, dose of intervention, compliance with the intervention and choice of the study population influence the interpretation of results. For instance, although adenoma

recurrence seems to be a valid surrogate endpoint for colorectal cancer, the concordance between the two endpoints is only modest. For instance, it is estimated that only 2.5 per 1000 adenomas evolve into cancer each year [57]. Also, four years of follow-up may be too short, when one considers the time course over which colorectal tumours evolve [58]. Moreover, it may also be possible that vegetables and fruits exert a protective effect at a later stage of carcinogenesis when the adenoma evolves into cancer.

Compliance with the intervention is oftentimes difficult to assess. Questionnaires as well as actual weight measurements [52] or assessment of urinary metabolites may not provide sufficient information.

Choice of study population remains an important issue. For instance, those with previous adenomas may not be representative of the population at large.

The appropriate dosage is difficult to assess. Possibly, in the Polyp Prevention Trial, an increase of fruits and vegetables of 66% may not be sufficient among a population with a habitual low level of intake at baseline. Alternatively, dosages may be too high, exceeding toxic levels.

Future perspectives

The question whether a diet rich in plant foods may protect against cancer is clearly evolving, but, certainly, further studies are necessary to obtain a definitive answer.

The use of questionnaires, lack of reliable databases for non-nutrients, confounding issues and selection of populations may limit observational studies. However, although randomised controlled trials are commonly regarded as providing more definitive support for causal inferences than observational studies these studies can usually answer only narrowly defined questions. Also, experimental studies cannot easily assess the effects of the long-term dietary patterns that have been shown to be associated with a lower risk of cancer in observational studies. Thus, with regard to plant foods, plant food constituents and cancer, though, definitive answers still seem to be beyond the reach of both observational epidemiologic studies as well as randomised controlled trials. Both types of studies could benefit by implementing methods and techniques used in genetics and molecular biology [59]. Alternative designs in observational studies, taking genetic susceptibility into account with adequate sample size should be evaluated [60]. New experimental designs are needed to study the effects of plant foods on the later stages of the development of cancer. Integrated approaches conducting studies on the cellular, individual and population level simultaneously may help to find useful and valid biomarkers of exposure and disease. Moreover, such an

approach may be most efficient and successful in unravelling the role of plant foods in carcinogenesis. It is, however, a challenging approach, which requires close collaboration between researchers of various disciplines, such as epidemiology, nutrition, biochemistry, genetics and molecular biology. This may at first be time-consuming and requires respect for other approaches and training across disciplines.

In the mean-time, it remains reasonable to recommend a high consumption of vegetables and fruits as part of a healthy diet to prevent cancer and other chronic diseases.

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