Mediterranean diet, antioxidants and cancer: the need for randomized trials

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In nutritional epidemiology the traditional approach has been to assess single nutrients or food items. Now, a growing interest exists in dietary patterns. The study of dietary patterns with a whole-diet approach represents a needed and complementary methodology. Among *a priori* defined patterns, the highly palatable traditional Mediterranean diet has many options to be the first choice in the dietary prevention of cancer. However, sound epidemiologic evidence about its ability to prevent the most frequent cancers is scarce. In conjunction with large and well-designed cohort studies, randomized trials using a whole-diet approach and not a simple antioxidant supplement are needed in Mediterranean countries. *European Journal of*

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Introduction

Cancer is a leading cause of morbidity and mortality in most developed countries. Cancer prevention is therefore an absolute priority for public health, and it should be the obvious choice for financial, social, and above all, human reasons (Giacosa *et al.*, 2003). Cancer and cardiovascular diseases are both known to be fairly refractory to curative interventions. In addition, the occurrence of these diseases exhibits a wide between-country variability and is largely determined by environmental factors and lifestyle habits. Primary prevention through diet and lifestyle (Stampfer *et al.*, 2000) is today an established reality to prevent coronary heart disease and stroke, and has achieved an important role in reducing the incidence of, and mortality from, cardiovascular diseases (Braunwald, 1997). However, attempts at prevention of cancer have thus far had very modest success (Vainio, 2000).

One of the potential answers to overcome this underachievement is related to the growing interest in the Mediterranean dietary pattern as a model for healthy eating and for the primary prevention of cancer. The incidence of certain cancers (e.g. breast and colon cancers) is (or it was) lowest in the Mediterranean area. In contrast with northern European and American diets, traditional Mediterranean diets provide large amount of plant foods. Ancient diets in Mediterranean countries were characterized by abundant consumption of fruits, vegetables, breads, nuts, seeds, wine and olive oil. All these items are important sources of dietary antioxidants. Apart from the most widely known antioxidants contained in fruits and vegetables, other compounds such as oleuropein, hydroxytyrosol and other polyphenols present in olive oil, and resveratrol, from red wine, possess a marked antioxidant activity and other advantageous biological properties. The role of free radical production in carcinogenesis together with many epidemiologic studies linking antioxidant intake with a reduced incidence of cancer indicates that dietary antioxidants probably play a protective role. Therefore, the highly palatable traditional Mediterranean diet has many options to be the first choice in the dietary prevention of cancer.

In this paper a critical review of the existing epidemiological evidence to support this hypothesis is done. Future lines of development for the next few years are also presented.

Eras and phases regarding exposure to antioxidants and the Mediterranean diet

In nutritional epidemiology the classical analytical approach to assess the causal effect of diet on health was to consider the exposure to single nutrients, mainly defined from a biochemical point of view. The isolated effect of each macronutrient (i.e. carbohydrates, protein or the different types of fat) was the main target of most published research during the 1960s, the 1970s and even the 1980s. During the late 1980s and early 1990s the interest shifted to micronutrients with a predominant emphasis on vitamins and antioxidants (carotenoids, tocopherols, vitamin C, selenium). More recently, specific food items (e.g. garlic, Fleischauer and Arab, 2001; olive oil, Martin-Moreno *et al.*, 1994; Fernandez-Jarne *et al.*, 2002; tomatoes, Giovannucci, 2002) have also attracted the attention of nutritional epidemiologists, because purified antioxidants or phytochemicals probably

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have lower ability to confer health benefits than the whole food or mixture of foods in which the antioxidants are present (Liu, 2003). The comprehensive analysis of the role of each possible isolated food item in the risk of a specific cancer creates huge problems. These problems relate to the impossibility of briefly reporting results and to statistical issues of multiple comparisons, findings by chance and 'fishing hypothesis'.

Although considerable advances in knowledge have been gained with studies focused in single nutrients or foods, methods that only consider isolated elements for assessing dietary habits may suffer from a reductionism view (i.e. we might have been studying the leaf but not the forest) (Jacobs and Steffen, 2003). For example, diets high in antioxidants tend to be high in fibre, whole grain and potassium but low in saturated fat. How can we be certain that a reported relationship with the risk for cancer is due to high fibre, high antioxidant or low saturated fat content? The extremely inter-related nature of dietary exposures demands creative new approaches that take into account food grouping, food scores and indexes. Thus, the grouping of all fruits in a single variable and the grouping of all vegetables in another is a simple solution that has provided interesting answers to cancer aetiology (Maynard *et al.*, 2003). Similarly new indexes such as the glycaemic index (Augustin *et al.*, 2002), the glycaemic load (Willett *et al.*, 2002), or the total antioxidant capacity (Halvorsen *et al.*, 2002) have been developed.

More interestingly, a growing interest currently exists in studying overall dietary patterns because food items and nutrients could have synergistic or antagonistic effects when they are consumed in combination. Overall patterns represent the current practices found in the assessed population and therefore provide useful epidemiological information (Jacques and Tucker, 2001). Recent epidemiological studies have evaluated the relationship between empirically identified dietary patterns and the occurrence of cancer (Slattery *et al.*, 1998; Terry *et al.*, 2001a,b; Chen *et al.*, 2002; Handa and Kreiger, 2002; Fung *et al.*, 2003a). In addition to empirically identified patterns, hypothesis-oriented patterns based on available scientific evidence for a specific disease are an attractive alternative (Schulze *et al.*, 2003).

A complementary approach is recommended nowadays. Thus, when a food pattern is found to be related to an outcome, some deeper thought is usually given to those hypotheses supporting those specific constituents of the food pattern that may be responsible for the association. To test these hypotheses, progressive assessments of increasingly small parts of the food pattern and ultimately of single biochemical compounds are needed. Starting with food patterns, then taking these larger units apart to isolate simpler pathways has been referred to as 'research from the top down' (Jacobs and Murtaugh, 2000). On the other hand, the previously termed reductionism approach, which begins with individual nutrients and puts them together to form a more complex picture of diet–health associations, might be called 'research from the bottom-up'. Both strategies are currently considered important and complementary (Jacobs and Steffen, 2003).

In the context of assessing the overall effect of the adherence to a dietary pattern, the Mediterranean diet, being ecologically associated with an advantageous life expectancy, has attracted considerable interest. The concept of the 'Mediterranean diet' was first studied by Keys and Grande (1957) as the traditional dietary pattern found in olive-growing areas of Crete, Greece and southern Italy in the late 1950s and early 1960s (Trichopoulou and Lagiou, 1997). With some variations, it is also found in many regions of Italy, Albania, Spain, France, Lebanon, Morocco, Portugal, Syria, Tunisia and Turkey. It is characterized by a high consumption of legumes, grains, fruit and vegetables, a moderate alcohol intake and a low to moderate consumption of meats and dairy products. Mono-unsaturated fatty acids are the main source of fat with olive oil as the principal component used to dress salads and to cook (Martinez-Gonzalez and Sanchez-Villegas, 2004). The evaluation of the potential role of the Mediterranean diet in cancer prevention fits well in this predominant paradigm of dietary patterns.

Design issues in the relationship between Mediterranean diet and cancer

Simultaneously with the above-mentioned evolution towards food groupings and eating patterns, a parallel development has happened in the research designs applied in nutritional epidemiology.

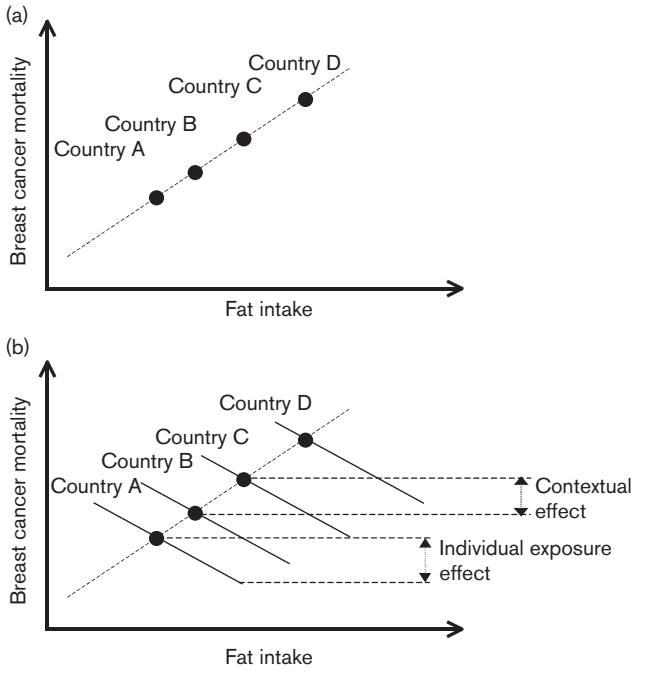
In the early days of nutritional epidemiology, the main sources of knowledge were observational investigations where the unit of analysis was not the individual, but a whole community or population. These are called ecological studies, and also cross-cultural studies or studies of international comparisons. In ecological studies, measurements of exposure routinely collected and aggregated at the household, local, district, regional, national or international level are compared with outcome measures aggregated at the same level. For example, when mortality rates for colon cancer in several countries or regions are plotted against the average intakes of saturated fat in these same countries an ecological study is being conducted (Martínez and Martínez-González, 2002).

Although ecological studies have sometimes been important in suggesting or generating new hypotheses, many flaws are likely to invalidate their findings. Perhaps

their best known limitation is the 'ecological fallacy' which is the bias resulting because an association observed between variables on an aggregated level does not necessarily represent the association that exists at an individual level. A positive correlation between total dietary fat intake (g/day) and risk of breast cancer mortality may be impressive when these data from some countries are plotted together, falsely suggesting that fat intake is the major determinant of breast cancer occurrence. This is an example of the misleading conclusions of an ecological study (Willett, 1998). To demonstrate this, we can see in Figure 1, panel a, that if an ecological study relating the average fat intake with breast cancer mortality finds a direct association, it would suggest that fat intake is a strong risk factor for breast cancer. However, these ecological results are fully compatible with the finding that at the individual level (i.e. within each country), women who consume more fat may exhibit a lower mortality from breast cancer.

Using this same figure, consider for a moment another example: average income (x axis) and body mass index (BMI, y axis). In fact this is what happens in reality: richer countries have a higher average BMI, but within each country richer people have a lower BMI (Diez-Roux *et al.*, 2002). In addition to this fallacy, and to many unmeasured confounders that are not usually taken into account, ecological studies measure diet less accurately because they use the average population intake as the exposure value for all individuals, which implies a high potential for biased assessment of diet–disease associations.

Fig. 1



Ecological and individual effects: the ecological fallacy. (a) Ecological association ('contextual effect'): an increased fat intake is interpreted as harmful. (b) Individual associations in the opposite direction (fat as protective) within countries are compatible with a direct effect at the ecological level.

Studies considering the individual (instead of the population) as the unit of observation are always preferable to avoid the flaws of ecological studies. Therefore, in spite of finding a lower cancer mortality or incidence in Mediterranean countries, international comparisons are not valid to establish the Mediterranean dietary pattern as a model for healthy eating to prevent cancer.

When we consider individual studies, one of the most frequently quoted articles supporting the protective role of diet on cancer is the paper by Block *et al.* (1992) who reviewed approximately 200 studies with individuals as the unit of analysis. These studies assessed the relationship between fruit and vegetable consumption and the occurrence of cancers of the lung, colon, breast, cervix, oesophagus, oral cavity, stomach, bladder, pancreas and ovary. In 128 of 156 dietary studies, the consumption of fruit and vegetables was found to have a significant protective effect. The risk of cancer for most cancer sites was twice as high in people whose intake of fruit and vegetables was low compared with those with high intake. However, many of these studies followed a case–control design. Case–control studies are by far the most logistically feasible of the analytical study designs in cancer epidemiology, but their application to nutritional exposures is limited by the particular nature of diet–disease relationships. The insight to be gained from a comparison of dietary exposures between cases and controls is limited by the possibility that dietary patterns of subjects have changed since the time that diet was most important to the disease process. This represents a severe problem because the relationship between cancer and diet is thought to have a long induction period.

To overcome this limitation, studies can be designed to measure past diet using food-frequency or diet history methods. Two major concerns are that recall of past diet by cases may be influenced by their present disease status, being different in cases from in controls (recall bias). The other concern is the difficulty in selecting adequate controls and in obtaining from them a high level of participation. Therefore, although some relevant case–control studies (Martin-Moreno *et al.*, 1994; Calza *et al.*, 2001; Fortes *et al.*, 2003; Bosetti *et al.*, 2003) have provided important findings suggesting the protective role of the Mediterranean dietary pattern on cancer, they also have inherent limitations because of their case–control design and, moreover, because these shortcomings are greater for

the nutritional epidemiology of cancer than for other associations. Therefore, stronger designs are needed.

The next step in the evolution of designs is the development of prospective cohort studies. Cohort studies are difficult to conduct and usually very expensive, because they require a prolonged and continuous follow-up of many thousands of participants during several years, usually for decades. Nevertheless, a substantial effort to develop large cohort studies in nutritional epidemiology has been done in the last two decades because they overcome the problems of recall bias and control selection.

Beyond the ecological associations and beyond the results of case–control studies, the adherence to a Mediterranean diet has in fact been consistently associated with a lower mortality from all causes also in some prospective cohort studies (Trichopoulou *et al.*, 1995, 2003; Kouris-Blazos *et al.*, 1999; Lasheras *et al.*, 2000). Furthermore, the largest of these studies has also reported a lower cancer mortality [adjusted hazard ratio, 0.76 (95% confidence interval 0.59–0.98)] associated with a higher adherence to an *a priori* defined Mediterranean dietary pattern (Trichopoulou *et al.*, 2003). However, these results only use mortality data and Mediterranean studies using incidence as the outcome are still lacking.

On the other hand, in the last 10 years, some contrasting results of observational studies with respect to those of subsequent large intervention controlled trials have raised major concerns. Observational studies consistently show that a predominantly plant-based diet reduces the risk for cancer, and it is often assumed that antioxidants (mainly carotenoids, vitamin E or vitamin C) contribute to this protection (Michaud *et al.*, 2000; Giovannucci *et al.*, 2002; Yuan *et al.*, 2003) but results from intervention trials (Table 1) with these antioxidants administered as supplements do not support major benefits (Lee, 1999).

The fear that important threats to validity may be present in large cohort studies has grown when discordant findings of observational and intervention studies regarding postmenopausal hormone use have been reported (Rossouw *et al.*, 2002; Grodstein *et al.*, 2003). However, recent reports from large and well-designed cohort studies have also failed to show a consistent overall protection for the best-known single antioxidants on cancer incidence for most cancer sites (Fairfield *et al.*, 2001; Jacobs *et al.*, 2001; Michels *et al.*, 2001; Zeegers *et al.*, 2001; Fung *et al.*, 2002, 2003b; Terry *et al.*, 2002; Wu *et al.*, 2002).

The answer to these paradoxes may be explained by the fact that plant foods contain many more compounds than the usually assessed antioxidants (carotenoids, vitamin C,

Table 1 Some randomized trials of antioxidants or dietary patterns and the risk of cancer (some other trials of vitamins/antioxidants with periodic updates can be found in Ritenbaugh *et al.*, 2003a)

Trial	Intervention	*N*	Main results/current status
Antioxidant supplements			
Factorial trials (2 × 2)			
PHS[a]	Beta carotene/Aspirin	22 071	No effect for beta-carotene
ATBC[a]	Alpha-tocopherol/Beta carotene	29 133	Beta-carotene: *higher* risk of lung cancer[*] Alpha-tocopherol: lower risk of prostate cancer[*]
APPS	Beta-carotene/Vitamin C + vitamin E	864	Beta-carotene protective against colon adenomas (only for non-drinkers, non-smokers)
SELECT	Selenium/Vitamin E	32 400	Results expected for 2013
WHS[a]	Beta carotene/Aspirin	39 876	No effect for beta-carotene (early termination)
Single intervention			
CARET[a]	Beta carotene + vitamin A	18 314	*Higher* risk of lung cancer
NPC	Selenium	1312	Lower risk of prostate cancer[*] *Higher* risk of non-melanoma skin cancer
MRC/BHF	Vitamin C + vitamin E + beta-carotene	20 536	No effect on cancer mortality
SUVIMAX	Vitamin C + vitamin E + beta-carotene + selenium + zinc	12 735	Results expected in 2004–2005
Whole dietary patterns			
PPS	Low-fat, high fibre	2079	No effect on recurrence of colon adenomas
WHEL	High-vegetable, low-fat diet	3088	Follow-up to be completed in 2006
WHI	High-fruit and vegetable, low-fat diet	48 836	Follow-up to be completed in 2007
Lyon D-H	Mediterranean diet	605	Inconclusive reduction in cancer (only 24 cases)
PREDIMED	Mediterranean diet	12 105	Follow-up to be completed in 2007

[*]Significant protection shown for at least one cancer site.
[a]See Figure 4 for description and references.
APPS, Antioxidant Polyp Prevention Study (Baron *et al.*, 2003); SELECT, Selenium and vitamin E Cancer Prevention Trial (Klein *et al.*, 2001); NPC, Nutritional Prevention of Cancer (Duffield-Lillico *et al.*, 2003a,b); MRC/BHF, British Heart Protection Study (Heart Protection Collaborative Group, 2002); SUVIMAX, Supplementation en VItamines et Minéraux AntioXydants (Hercberg *et al.*, 1998); PPS, Polyp Prevention Study (Schatzkin *et al.*, 2000); WHEL, Women's Healthy Eating and Living (Pierce *et al.*, 2002); WHI, Women's Health Initiative (Ritenbaugh *et al.*, 2003b); Lyon D-H, Lyon Diet-Heart Study (De Lorgeril *et al.*, 1998, 1999); PREDIMED, Spanish network of investigators about diet and cardiovascular disease. Trial with three groups: (a) low-fat diet (control); (b) Mediterranean diet + supply of extra-virgin olive oil; and (c) Mediterranean diet + supply of nuts. Further information available from the Coordinator of the Project (Prof Ramon Estruch, RESTRUCH@clinic.ub.es) or from the author of this article (mamartinez@unav.es).

vitamin E, selenium). Many phytochemicals have been shown to be biologically active and they may interact to protect against cancer. For example, mechanistic data suggest that the flavonoids, a group of more than 4000 polyphenolic antioxidant components of fruits and vegetables, may contribute to cancer prevention. They have been found to be protective in some observational studies (Hertog *et al.*, 1994; Knekt *et al.*, 1997, 2002; Le Marchand *et al.*, 2000; Hirvonen *et al.*, 2001; Arts *et al.*, 2002), although further epidemiological evidence is needed, because not all studies show consistency (Garcia *et al.*, 1999; Arts *et al.*, 2001). Wild edible greens frequently eaten in rural Mediterranean areas, mainly in the form of salads, are very rich in flavonoids (Trichopoulou and Vasilopoulou, 2000). It is more likely that the full synergistic effects of phytochemicals may account for their antioxidant and anticancer activities. Thus, the advantages for cancer prevention of a diet rich in fruit and vegetables is more closely related to the complex mixture of phytochemicals present in whole foods (Liu, 2003) than to the provision of some antioxidants in a pill.

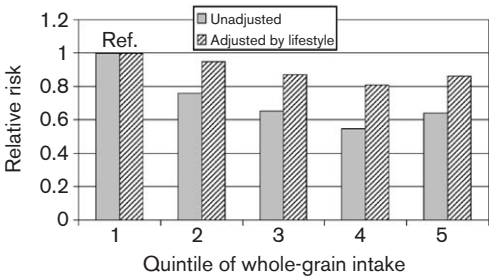
This view is increasingly achieving a wide consensus because it may explain why no single antioxidant can replace the combination of natural phytochemicals in fruit and vegetables. There are more than 8000 phytochemicals present in whole foods. These compounds differ in solubility, molecular size and polarity, characteristics that may affect their bioavailability and their biological properties in cells, organs and tissues. A supplement given as a pill simply cannot mimic this balanced natural combination of bioactive compounds present in fruit, vegetables, extra-virgin olive oil and red wine (Jacobs and Murtaugh, 2000; Visioli and Galli, 2002; Jacobs and Steffen 2003; Liu, 2003).

On the other hand, cohort studies, being observational in design, do not assign participants to dietary exposures. Participants instead are free to choose their own diet according to their particular preferences, while in trials participants are randomly allocated to follow the diet assigned by the investigator. A suspicion has been raised about the validity of cohort studies because cohort participants who are otherwise healthier (non-smokers, physically active, more health conscious, etc.) are more likely to self-select themselves to follow what is socially considered as a 'healthy diet'. This means that in cohort studies, as in any observational study, the effect of potential confounding factors needs to be thoroughly considered. The usual approach is to measure other aspects of lifestyle and diet and to control for them using multivariate analyses. This is particularly important for analyses of antioxidant supplement use because vitamin/antioxidant users may be more likely to have health conscious behaviours. Users of antioxidant supplements may differ in their lifestyle to a great extent from non-users and not all of these differences can be fully accounted for in the multivariate analyses. The same could be said of fruit and vegetable consumption or of whole-grain intake. The question then is what proportion of the protection observed in cohort studies is actually due to dietary exposures and what proportion to lifestyle?

Looking at Figure 2, we can see that when other aspects of lifestyle are taken into account, the apparent protection conferred by whole-grain consumption, substantially decreases. Whole-grain consumption is a nutritional exposure socially considered as 'healthy' and it is more prevalent among non-smokers, physically active subjects and otherwise healthier people. As our ability to adjust for every aspect of lifestyle and diet in observational epidemiology is limited because of the difficulty of accurately collecting and measuring all these aspects, it is plausible that even in the multivariate adjusted analysis, some other aspects of diet and lifestyle are not accounted for, and residual confounding still remains in these estimates. Attempts by epidemiologists to analyse observational cohort studies in ways that replicate, to the extent possible, clinical trial designs would be extremely useful in elucidating the sources of the differences in findings between clinical trials and cohort studies (Ritenbaugh *et al.*, 2003a). In any case, the advantage of rigorously conducted randomized trials is that they are able to adjust for measured and unmeasured confounders. Understanding the sources of their differences will permit us to use the cohort study data more effectively and to improve the design of long-term clinical trials with cancer events as the outcome.

In addition to the problem of residual confounding, some unavoidable degree of measurement error is always present in observational cohorts (Michels, 2001). The transformation of food consumption data collected by food-frequency questionnaires into actual nutrient intake

Fig. 2



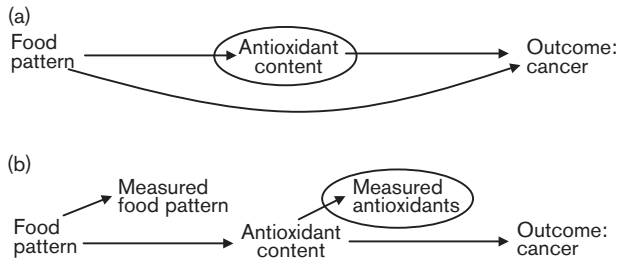
Differences in estimates of relative risk associated with whole-grain intake when lifestyle is taken into account (adjusted estimates). Relative risks of total mortality, Iowa Women cohort (Jacobs *et al.*, 1999). Unadjusted: adjusted only for age and total energy intake. Adjusted: additionally adjusted for consumption of other plant foods, consumption of meats, smoking, physical activity, education and body fatness.

involves a multitude of problems. Foods are difficult to measure accurately. The accurate measurement of phytochemicals is even more difficult. A certain degree of inaccuracy is present in the application of the database of nutrients, both in its catalogue of relevant foods and in its imputation of nutrient content.

Sometimes in observational studies a particular food pattern shows a protection against cancer. Mechanistic reasons lead to the assumption that this protection may be due to the high antioxidant load provided by this pattern, but adjustment for a myriad of antioxidants does not explain the protection afforded by the pattern. The simplistic and naïve interpretation of this fact is that 'something else in the overall dietary pattern protects against cancer' (see causal diagram, Greenland *et al.*, 1999; Figure 3a). This interpretation has been commonly given to this kind of finding in nutritional epidemiology, but this explanation does not need to be true. Figure 3b shows another causal diagram that can alternatively explain the situation. Even if all the effect of the dietary pattern were mediated through its antioxidant content, the statistical adjustment for antioxidants would not fully explain the association, because the measured antioxidants do not correspond to the total antioxidant content of that food pattern.

The use of experimental designs such as large intervention trials helps to overcome not only residual confounding but also most problems of measurement error, because the dietary exposure in a trial is not merely observed or measured, but assigned by the investigator, who therefore can accurately control it. Thus, intervention trials are the needed gold standard for causal inference in nutritional epidemiology.

Fig. 3



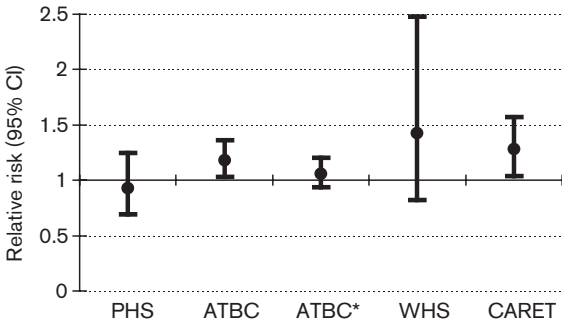
Causal diagrams. Measurement error (e.g. inaccuracy of food composition tables) may account for antioxidants not explaining the protection afforded by a dietary pattern. (a) Naïve and simplistic explanation: if the association persists after adjustment for antioxidants, something else in the pattern may account for its protection against cancer. (b) Realistic explanation: the adjustment for antioxidant content is not perfect because they are always inaccurately measured. The circles around variables indicate the adjustment or controlling for those variables by means of stratification or statistical modelling.

Randomized trials of antioxidants and Mediterranean diets

The development of dietary trials to prevent cancer has had a discouraging start. Case–control and cohort studies had almost consistently found that the risk of the most frequent cancers was inversely related to the consumption of green and yellow vegetables and fruit. Because beta-carotene is present in abundance in these plant foods, it was thereafter investigated as a possible cancer-preventive agent using the single-nutrient approach. Thus, early observational studies suggested that beta-carotene was preventive against cancers of the lung, oral cavity and pharynx. But the initial enthusiasm for beta-carotene almost entirely disappeared with the unexpected results of two large trials that rigorously assessed this issue. The Finnish Alpha-Tocopherol, Beta-Carotene (ATBC) Study found that beta-carotene (20 mg/day) significantly increased the risk of lung cancer (Alpha-Tocopherol, Beta Carotene Cancer Prevention Study Group, 1994) and the American Beta-Carotene and Retinol Efficacy Trial (CARET) also reported that beta-carotene (30 mg/day) administered with retinol (25 000 IU/day) increased the risk of lung cancer (Omenn *et al.*, 1996). Figure 4 shows the results of good-quality trials of beta-carotene in the prevention of lung cancer. In fact, substantial data are currently available to conclude that beta-carotene supplementation actually increases the risk of lung cancer.

Still more discouraging is that subsequent randomized trials using single antioxidants (or combinations with a few of them) generally have not supported the protective

Fig. 4



Randomized trials of beta-carotene supplementation. Relative risks of lung cancer. PHS, Physicians Health Study (50 mg on alternate days), 22 071 US male physicians (Hennekens *et al.*, 1996). ATBC, Alpha-Tocopherol, Beta Carotene Cancer Prevention Study (20 mg/day), 29 133 Finnish male smokers (Alpha-Tocopherol, Beta Carotene Cancer Prevention Study Group, 1994). ATBC*, Postintervention follow-up of the ATBC trial (Virtamo *et al.*, 2003). WHS, Women's Health Study (50 mg on alternate days), 39 876 US female health professionals (Lee *et al.*, 1999). CARET, Beta-Carotene and Retinol Efficacy Trial (30 mg/day plus vitamin A), 18 000 high-risk participants (asbestos workers and heavy smokers) (Omenn *et al.*, 1996).

hypothesis (Table 1). Several explanations for the inconsistent findings between observational results and intervention trials have been given. In addition to the above-mentioned possibility of residual confounding in observational studies, these interpretations include: (1) the short duration of follow-up in most randomized trials relatively to the long induction period expected for diet–cancer associations, and (2) the protective role of a combination of many different nutrients present in fruits and vegetables, rather than the single nutrient or combination of two nutrients that most trials have tested. It is now believed that dietary supplements do not offer the same health benefits as a diet rich in fruit and vegetables because, taken alone, the isolated pure compound either loses its bioactivity or may not behave the same way as the compound in whole foods (Vainio, 2000; Jacobs and Steffen, 2003; Liu, 2003). This explanation is very likely to shape the future in the design of diet–cancer studies and converges with the paradigm of food patterns and the refutation of the reductionism view in nutritional epidemiology. A recent report with a re-analysis of the data from the ATBC trial suggests that high fruit and vegetable consumption in the usual diet, particularly a diet rich in carotenoids, tomatoes and tomato-based products, may reduce the risk of lung cancer, but supplementation with beta-carotene increased this risk (Holick *et al.*, 2002).

Most randomized trials have used and are still using the single-nutrient reductionism approach. However, some intervention trials did adopt a whole-pattern design for the experimental diet. Table 1 shows selected large intervention trials classified in two groups depending on the use of an isolated-nutrient approach (supplements) or the use of a whole-diet approach. Based on the available evidence, it seems prudent to advocate a whole-diet approach, rich in fruits and vegetables, rather than the consumption of specific antioxidant vitamin supplements, in order to decrease the risk of developing cancer. In view of this the scarcity of available results from large trials using the dietary pattern approach seems surprising.

Clearly it is easier to test single antioxidants in randomized trials, but the association between dietary antioxidants and health may be difficult to ascertain if hundreds of carotenoids, polyphenolic acids, sulphides, flavonoids, lignans, and so on are bioactive and work synergistically. Complementary studies of food and food patterns and of nutrients and specific food constituents will enhance the understanding of the role of antioxidants in Mediterranean diets. The need to assess overall dietary patterns has already arrived to the field of observational epidemiology and it is rendering abundant results, but this approach is materially absent in experimental trials. Unless these trials are soon started the required evidence will not be available in a very long time.

However, intervention trials also present some drawbacks, because they can examine only a narrow spectrum of dietary exposures and only within a narrow time frame between exposure and outcome. The time frame for cancer prevention may be longer than the follow-up period in the trials. In addition, they usually lack generalizability. Hence, epidemiological cohort studies will continue to be extremely important in providing guidance regarding the role of dietary patterns and antioxidants in the prevention of chronic disease. Observational cohort studies and intervention trials should be used in conjunction as complementary tools. Several important cohorts in nutritional epidemiology such as the Nurses' Health Study have been already followed-up for a long time and are now able to provide information on risks and benefits associated with dietary exposures taking place early in the carcinogenesis process (Ritenbaugh *et al.*, 2003a). In this aspect a cohort may overcome the advantages of a trial. For example, the results of the Boyd Orr cohort (Maynard *et al.*, 2003) with over 60 years follow-up will be almost impossible to confirm or refute by a randomized trial.

Similar to what has already been said at the beginning of this article about the complementary nature of the 'research from the top down' and 'from the bottom-up', cohorts and trials do not represent mutually exclusive options, but complementary approaches.

The need for Mediterranean trials

The usual option in the design of a whole-pattern trial has been to select a low-fat diet, but the traditional Mediterranean diet was rich in fat. Most trials with dietary patterns have only tested low-fat diets. The only exception is the Lyon Trial (De Lorgeril *et al.*, 1998). This study was designed for secondary prevention of myocardial infarction and assigned patients to an experimental diet rich in bread, vegetables, fish and fruit and low in red meat (replaced with poultry). Butter and cream were replaced with a special margarine rich in alpha-linolenic acid (De Lorgeril *et al.*, 1999). The published results of the Lyon trial with respect to cancer are materially inconclusive because only 24 cases of cancer were observed (De Lorgeril *et al.*, 1998). Therefore, no available trial with a Mediterranean-type high-fat diet for cancer prevention exists.

In spite of its relatively high fat content, the theoretical advantages of the Mediterranean diet are multiple. The high fruit and vegetable intake adds to its high antioxidant load and other mechanistic benefits provided by the consumption of extra-virgin olive oil and red wine. Furthermore, the Mediterranean diet represents a considerably more palatable alternative than the usual low-fat approaches for promoting healthy eating. Hence, in intervention trials a higher compliance with

Mediterranean diet will be expected. The strong background of a long tradition with no evidence of harm, also makes the Mediterranean diet very promising for public health nutrition.

All the previous considerations represent a strong rationale for starting intervention trials to test this dietary pattern rigorously. Not all of its components have to be protective, or at least, they may not provide the same degree of protection, and a need exists to identify those that are mainly responsible for the purported effect. Specifically designed primary prevention trials with incident cancer events as the outcome carried out in the Mediterranean region in conjunction with cohort studies are especially suited to prove these potential benefits.

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