Fruits and vegetables and the risk of epithelial cancer

Margje CJF Jansen

Promotoren
Prof. dr ir D Kromhout
Hoogleraar Volksgezondheidsonderzoek
Wageningen Universiteit

Prof. dr ir FJ Kok
Hoogleraar in de Voeding en Epidemiologie
Wageningen Universiteit

Co-promotor
Dr HB Bueno de Mesquita
Projectleider Kankerepidemiologie
Rijksinstituut voor Volksgezondheid en Milieu, Bilthoven

Promotiecommissie
Prof. dr ir PA van den Brandt
Universiteit Maastricht

Prof. dr WMF Jongen
Wageningen Universiteit

Prof. dr R Kroes
Universiteit Utrecht

Dr E Riboli
International Agency for Research on Cancer
Lyon, France

Dr ir P van 't Veer
Wageningen Universiteit

Fruits and vegetables and the risk of epithelial cancer

Margje CJF Jansen

Proefschrift
Ter verkrijging van de graad van doctor
op gezag van de rector magnificus
van Wageningen Universiteit
Prof. dr ir L Speelman
in het openbaar te verdedigen
op vrijdag 12 oktober 2001
des namiddags te vier uur in de Aula.

The research described in this thesis was conducted at the Department of Chronic Diseases Epidemiology of the National Institute of Public Health and the Environment in Bilthoven in collaboration with the Division of Human Nutrition and Epidemiology of Wageningen University.

The PhD-project of Margje CJF Jansen was carried out in the framework of the Graduate Schools VLAG (Food Technology, Agrobiotehnology, Nutrition and Health Sciences) and NIHES (Netherlands Institute for Health Sciences).

Jansen, Margje Carola Johanna Francisca
Fruits and vegetables and the risk of epithelial cancer
Thesis Wageningen University – With references – With summary in Dutch

ISBN 90-5808-507-4

Printed by Universal Press, Veenendaal, The Netherlands

Stellingen

1. Variatie in groenteconsumptie verlaagt het risico op kanker.

2. Eva *snoepte verstandig.*

3. Een hoog absoluut risico is de beste uitgangssituatie voor een grote relatieve risicoreductie.

4. 'De rol van genen wordt ook nogal eens overschat omdat vooral heel dominante afwijkingen in heel specifieke families zijn bestudeerd. De alledaagse rol van de genen, die moeten we nog leren begrijpen.'
   Prof dr D Boomsma, Volkskrant 1 september 2001

5. Een onderzoeker die wetenschappelijke bevindingen op het gebied van voeding en gezondheid in de media brengt, dient zich bewust te zijn dat de kortstondige aandacht niet bijdraagt aan gedragsveranderingen op langere termijn.

6. Cohortonderzoek is gebaat bij grote aantallen kankerpatiënten; hierbuiten is elk geval er één teveel.

7. Uit de term welvaartsziekten kan afgeleid worden dat ook welvaart een optimum kent.

8. Kijken over de grenzen verscherpt je blik op je eigen gebied.

Stellingen behorend bij het proefschrift 'Fruits and vegetables and the risk of epithelial cancer'.

Margje CJF Jansen
Wageningen, 12 oktober 2001

Aan mijn vader,
Koos Jansen

Abstract

In this thesis, prospective studies on fruit and vegetable consumption in relation to epithelial cancer risk were described. The main research question was whether higher intakes were related to lower risks of epithelial cancers, mainly of lung cancer.
In the Seven Countries Study, at the population level, consumption of fruits, vegetables and total plant foods was not related to colorectal cancer risk, whereas a difference of 10 g/d of fiber intake was associated with a 33% lower risk. Average population consumption of fruit was inversely and of refined grains positively related to population stomach cancer risk. Low consumption of fruits was however strongly correlated with high refined grain consumption.
Fruit but not vegetable consumption was inversely associated with 25-year lung cancer mortality among European smoking men. This association was confined to heavy cigarette smokers. In Dutch men and women aged 20-59, vegetable consumption was inversely associated with lung cancer incidence, particularly of adenocarcinomas. Fruit consumption was not related to lung cancer after adjustment for smoking.
Adherence to the dietary guidelines for fruits and vegetables was inversely associated with cancer incidence in Dutch elderly men. Consumption of the recommended amount of fruit was related to a 38% lower risk, while vegetables were not associated. Variety in vegetable intake was however inversely related to total and non-lung epithelial cancer.
Plasma carotenoid concentrations were only crude indicators of usual vegetable and fruit intake in Dutch men and women aged 20-59. Plasma β-cryptoxanthin indicated fruit intake and total intake of vegetables, fruits and juices, whereas lutein was a marker for vegetable intake. Concentrations of carotenoids could not differ between all four quartiles of intake.
During 1987/88-1997/98, the mean fruit and vegetable consumption (excluding juices) decreased with 34 g/d (12%) in Dutch men and 23 g/d (8%) in Dutch women. The consumption was lowest and decreased most in those aged 19-35 with a low level of education. Using a computer simulation model, the maximum theoretically reduction in cancer incidence, i.e., when all would consume the recommended 400 grams daily, was estimated to be 14 to 22% for this group.
Valid assessment of fruit and mainly of vegetable intake, residual confounding by smoking and enough power of the study are major methodological concerns. In recent cohort studies weaker associations were observed compared to earlier risk estimates. Taken all evidence together, an inverse association between fruit and vegetable intake and cancer of the lung, stomach and colon/rectum is still indicated. There is not enough evidence yet to point at specific fruits and vegetables or plant compounds as responsible actors.

Contents

Chapter 1

General introduction

Cancer occurrence

Cancer is a major health problem throughout the world, indicated by the cancer incidence of 10 million cases worldwide in 2000[1]. In 1997, 65,000 cancer cases of which 34,000 in men were diagnosed in The Netherlands[2]. Because 10% of these cases were already prevalent cancer cases, cancer incidence was around 58,500 cases. In men, lung cancer was most common (20%), followed by prostate cancer (19%) and colorectal cancer (13%). In women, breast cancer occurred most (32%), followed by colorectal cancer (13%) and lung cancer (7%). Stomach cancer comprised 4% of the male cancer cases and 2% of the female cases. Cancer is most prevalent in older individuals: 66% of all women and 75% of all men with cancer were aged 60 years and over. In 1997, cancer was the second cause of death in The Netherlands with 37,000 cases. The prognosis of lung cancer and stomach cancer is poor. The percentage of patients dying within 5 years is 85-95% and 80-90%, respectively. For colorectal cancer, prognosis is somewhat better with this percentage being 40-50[2].

Diet, smoking and cancer

Cancer incidence varies widely around the world and over time[3]. This fact combined with observations that rates in immigrants converge to local cancer rates within a few generations[4] exclude the possibility that variation in cancer patterns is fully due to genetic differences.

Based on ecological, time trend and migration studies, Doll and Peto estimated in 1981 that dietary modification could potentially avoid 35% of the US cancer deaths. The range around their estimation was wide, i.e., 10 to 70%[5], and was narrowed to 20-60% by Doll ten years later[6]. Doll and Peto stressed the need for analytical epidemiological studies investigating the role of diet in the occurrence of cancer[5]. Such studies have indeed been performed, and based on these results Willett reduced the uncertainty of Doll and Peto to a range of 20 to 42% with 32% as best estimate in 1995[7].

Whereas in earlier thinking about the relation between diet and cancer especially adverse effects were emphasized, such as the association high fat in relation to colorectal cancer, epidemiological studies conducted since the eighties yielded accumulating evidence for a protective effect of fruits and vegetables on epithelial cancer risk.

The carcinogenic effect of smoking tobacco is beyond dispute. In 1981, Doll and Peto estimated smoking to be responsible for one-third of the cancer cases[5]. This is still the estimation for developed countries, and among smokers 60% of the cancers are contributed to smoking[8]. Especially the risk of lung cancer is predominantly

determined by smoking; the estimated attributable risk is 80-90% in men and 60-80% in women[9]. Also other forms of cancer, among them stomach cancer and colorectal cancer have been related to smoking[9,10]. Most evidence exists for cigarette smoking, however, studies show that also cigar and pipe smoking raises lung cancer risk[11,12]. Twenty carcinogens in tobacco smoke convincingly cause lung tumors in laboratory animals or humans. Of these, polycyclic aromatic hydrocarbons (PAHs) such as benzo[a]pyrene, and 4-(methylnitrosamino)1-(3-pyridyl)-1-butanone (NNK) are likely to play major roles. These carcinogens require metabolic activation. Detoxification differs among individuals, and this will affect cancer risk[13].

Because of the tremendous impact of smoking on cancer and because smokers tend to eat less fruit and vegetables[14], residual confounding of smoking in the observed relation between fruit and vegetable intake and cancer is a serious possibility. Therefore, adjustment for smoking in the analysis is of major importance.

Fruit and vegetable intake in relation to epithelial cancer

At the start of the research described in this thesis, many epidemiological studies on the association between fruit and vegetable intake and cancer were already conducted. Studies investigated both intakes of whole foods as their components. Many studies indicated an inverse association, although statistical significance was not always reached. Risk reductions were most consistent and greatest in magnitude for epithelial cancers of the respiratory and alimentary tracts. Several papers systematically reviewed the existing evidence[15,16]. Because the majority of the conducted studies were case-control studies, which are prone to several types of biases, the question was raised whether these observations would hold in prospective studies. Other unsolved issues were inconsistent results for subgroups within populations, for example between smokers and non-smokers. Moreover, it was not clear whether specific fruits or vegetables, subgroups or components were responsible for the inverse associations. Grains, especially whole grains, were also postulated as plant foods that potentially could prevent cancer[17].

Therefore, in this thesis epithelial cancers, mainly lung cancer, are taken as endpoints of interest in prospective epidemiological studies. Besides the overall effect of fruit and vegetable intakes, subgroups of these foods are related to cancer risk, and populations are divided into subgroups whenever relevant and numbers are large enough. In these subgroup-analyses it is tried to address etiological questions still remaining in this research area.

Carcinogenesis and proposed protective mechanisms

Cancer is characterized by DNA damage leading to unprogrammed and uncontrolled cell proliferation[18]. Evidence is growing that cancer is a result of an accumulation of genetic damage. This basic concept is common to all cancers, but cancer cannot be seen as a single disease. Cancers are derived from numerous tissues with multiple etiologies. The causes of cancer are many and varied, and include genetic predisposition, environmental influences, lifestyle factors and infectious agents[19].

Carcinogenesis is a multi-stage process and can be simplified by the following steps: exposure to relevant agent(s), metabolism of agent(s), interaction between agent(s) and DNA (i.e. initiation), repair of DNA damage, apoptosis or persistence and replication of transformed cells, growth of transformed cells into pre-neoplastic cells (i.e. promotion), further growth of tumor cells and spread to other parts of the body (i.e. progression). The time between initiation and clinical signs of cancer can be decades. Inherited abnormalities in DNA are rare and most DNA damages occur during life. Genetic damage can also be induced by normal metabolic functions producing oxygen radicals. Mutations may induce oncogenes and limit functioning of tumor suppressor genes and DNA-repair genes. Genes may also lose their function by for example hyper- or hypomethylation[20].

It is suggested that cancers result from six essential alterations in cell physiology that lead in combination to malignant growth, i.e., self-sufficiency in growth signals, insensitivity to growth-inhibitory signals, evasion of apoptosis, limitless replicative potential, sustained angiogenesis, and tissue invasion and metastasis[18].

Not all DNA damage results in cancer. DNA-repair mechanisms and apoptosis may prevent initiated cells from growing into cancer cells. Moreover, not all mutations come to expression, and thus do not lead to functional changes. Alternatively, exogenous factors may protect against cancer by interacting with the carcinogenic process. Fruits and vegetables contain many compounds for which protective effects have been postulated or shown experimentally. Proposed preventive mechanisms include their antioxidant activity, modulation of detoxification enzymes, stimulation of the immune system and antibacterial and antiviral activity[21].

Formation of carcinogenic agents may be prevented by plant compounds, such as the formation of nitrosamines which is thought to be inhibited by vitamin C. Plant compounds such as vitamin C and E can scavenge oxygen radicals before they can damage DNA; this is also called antioxidant activity. Moreover, most carcinogens need activation before they can potentially damage DNA. Activation is a normal enzymatic process and is performed by phase I enzymes, such as cytochrome P450. Thereafter, carcinogens can be detoxified by phase II enzymes. Components from fruits and vegetables are hypothesized to prevent cancer through modulation of

phase I and II enzymes activities. For example, carotenoids are hypothesized to block the phase I enzymes and isothiocyanates in cabbages are hypothesized to induce phase II enzymes. Plant food compounds may also exert their action later in the cancer process, such as stimulating apoptosis or suppressing cellular proliferation. Several mechanisms have been postulated for suppression, among them are antioxidation, improvement of cell communication and stimulating immune factors[22,23]. Several compounds from fruits and vegetables have been hypothesized to possess anticarcinogenic effects, while many compounds present in these foods have not been identified yet.

Alternatively, it is still possible that associations between vegetable and fruit intake and cancer are not causal. Till now no ultimate proof is present. Other characteristics of individuals associated with eating fruits and vegetables may explain the associations observed. Smoking is the main candidate in this context. However, dietary and other lifestyle factors are not equally associated in different societies, and yet the inverse relation between intake of fruits and vegetables has been observed throughout the world.

Assessment of fruit and vegetable intake

One of the weaknesses of observational epidemiological studies is the difficulty of assessing intake of fruits and vegetables in a valid and precise way[24]. Especially measuring usual intake, which is of major importance when studying relations with cancer risk, is difficult. Seasonal variation in intake and assessment of portion sizes are specific problems in assessing fruit and vegetable intake.

Measuring fruit intake seems less problematic compared to vegetables. The main reasons for this difference are that in general only a few fruits contribute most to total fruit intake, and that fruit consumption can often be estimated in pieces or bowls. The variety in vegetables eaten is mostly much greater and consequently results in less frequent consumption of each type, making estimation of the average consumption per vegetable more difficult.

In the studies described in this thesis, several dietary assessment methods have been used, i.e., a record method in Chapters 2, 3 and 8, collecting food composites in Chapter 2 and 3, a cross-check dietary history in Chapters 4 and 6, and food frequency questionnaires in Chapters 5 and 7.

Given the potential measurement error in the assessment of fruit and vegetable intake, biochemical indicators, i.e. biomarkers, of intake for these foods could be useful in epidemiological research. Carotenoids are under study for this purpose.

Outline of thesis

In this thesis intakes of fruits and vegetables are associated with the risk of epithelial cancers, in a number of populations using several dietary assessment methods. In addition, some attention has been paid to the role of intakes of grains, potatoes and legumes, i.e., other plant food groups. All etiological studies conducted were prospectively designed, in order to test whether such studies resulted in less consistent or less strong relationships between fruit and vegetable intake and cancer risk compared to case-control studies. Moreover, subgroups of intake and dietary components were associated with cancer to investigate whether specific groups or components could be responsible for cancer prevention. Plasma carotenoid concentrations were studied cross-sectionally for their potential to categorize persons according to usual fruit and vegetable intake. To place the research in a public health perspective, the potential impact of fruits and vegetables on reducing cancer incidence in a sub-population in The Netherlands was estimated by computer simulation.

Prospective studies were conducted at the population level (Chapters 2 and 3) and at the individual level (Chapters 4, 5 and 6). Intakes of fruits, vegetables, other plant food groups, their subgroups and several compounds were studied in relation to colorectal cancer mortality in Chapter 2 and stomach cancer mortality in Chapter 3. Both analyses were based on the international Seven Countries Study. In Chapter 4, the scope is restricted to Europe, i.e., cohorts from Finland, Italy and The Netherlands. In this study intakes of fruits and vegetables were related to lung cancer mortality in men aged 50-69, followed from 1970 to 1995. Chapter 5 refers to more recent Dutch data and also includes women. Fruit and vegetable intakes were associated with lung cancer incidence in Dutch men and women 20-59 years of age followed for 8.4 years on average.
In Chapter 2 to 5, intakes were divided into categories, such as mostly done in epidemiological research. However, dietary recommendations for fruit and vegetable intake have been formulated in absolute amounts. In The Netherlands it is advised to eat 200 grams of fruits and 150-200 grams of vegetables each day. Moreover, variety in intake of these foods is recommended. In Chapter 6 we therefore investigated whether adherence to these guidelines was associated with a lower cancer risk in elderly men from the Zutphen Elderly Study followed for 10 years.
Because assessment of fruit and vegetable intake has several drawbacks, we measured plasma carotenoid concentrations and related them to intake data in a sample of the MORGEN-project, one of the Dutch contributions to the EPIC-study (Chapter 7). In this cross-sectional study we investigated whether plasma carotenoid concentrations could be used to divide persons based on their usual fruit and vegetable intake. In Chapter 8 we briefly described the fruit and vegetable intake of

adults in The Netherlands based on the Dutch National Food Consumption Surveys held in 1987/88, 1992 and 1997/98. With the situation in 1997/98 as a starting point, we estimated the potential impact of increasing fruit and vegetable intake on the prevention of cancer incidence. Finally, a general discussion follows in Chapter 9.

References

1. Parkin DM, Bray FI, Devesa SS. Cancer burden in the year 2000. The global picture. Eur J Cancer (special supplement; in press).
2. Incidence of cancer in The Netherlands 1997. Visser O, Coebergh JWW, Schouten LJ, Dijck JAAM van (Eds.). Utrecht: Association of comprehensive cancer centres, 2001.
3. Parkin DM, Whelan SL, Ferlay J, Raymond L, Young J. Cancer incidence in five continents, volume VII, IARC Scientific Publications No. 143. Lyon, France: IARC; 1997.
4. Wynder EL, Shigematsu T. Environmental factors of cancer of the colon and rectum. Cancer 1967; 20: 1520-1561.
5. Doll R, Peto R. The causes of cancer: quantitative estimates of avoidable risks of cancer in the United States today. J Natl Cancer Inst 1981; 66 :1191-1308.
6. Doll R. The lessons of life: keynote address to the Nutrition and Cancer conference. Cancer Res 1992; 52(Suppl): 2024-2029.
7. Willett WC. Diet, nutrition, and avoidable cancer. Environ Health Perspect 1995; 103 (Suppl 8): 165-170.
8. Peto R, Lopez AD, Boreham J, Heath C, Thun M. Mortality from tobacco in developed countries 1950-2000. Oxford: Oxford University Press, 1994.
9. Tomatis L, Aitio A, Day NE, Heseltine E, Kaldor J, Miller AB, Parkin DM, Riboli E. (Eds.). Cancer: Causes, occurrence and control. IARC Scientific Publications No. 100. Lyon, France: IARC, 1990.
10. Peto J. Cancer epidemiology in the last century and the next decade. Nature 2001; 411: 390-395.
11. Boffetta P, Pershagen G, Jöckel K-H, Forastiere F, Gaborieau V, Heinrich J et al. Cigar and pipe smoking and lung cancer risk: a multicenter study from Europe. J Natl Cancer Inst 1999; 91: 697-701.
12. Shapiro JA, Jacobs EJ, Thun MJ. Cigar smoking in men and risk of death from tobacco-related cancers. J Natl Cancer Inst 2000; 92: 333-337.
13. Hecht SS. Tobacco smoke carcinogens and lung cancer. J Natl Cancer Inst 1999; 91: 1194-1210.
14. Morabia A, Wynder EL. Dietary habits of smokers, people who never smoked, and exsmokers. Am J Clin Nutr 1990; 52: 933-937.
15. Steinmetz KA, Potter JD. Vegetables, fruit, and cancer. I. Epidemiology. Cancer Causes Control 1991; 2: 325-357.
16. Block G, Patterson B, Subar A. Fruit, vegetables, and cancer prevention: a review of the epidemiological evidence. Nutr Cancer 1992; 18: 1-29.
17. Jacobs DR Jr, Slavin J, Marquart L. Whole grain intake and cancer: A review of the literature. Nutr Cancer 1995; 24: 221-229.
18. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell 2000; 100: 57-70.

19. Evan GI, Vousden KH. Proliferation, cell cycle and apoptosis in cancer. Nature 2001; 411: 342-348.
20. World Cancer Research Fund/American Institute for Cancer Research. Food, nutrition and the prevention of cancer: a global perspective. Washington, DC: American Institute for Cancer Research, 1997.
21. Lampe JW. Health effects of vegetables and fruit: assessing mechanisms of action in human experimental studies. Am J Clin Nutr 1999; 70(Suppl): S475-S490.
22. Steinmetz KA, Potter JD. Vegetables, fruit, and cancer. II. Mechanisms. Cancer Causes Control 1991; 2: 427-442.
23. Morse MA, Stoner GD. Cancer chemoprevention: principles and prospects. Carcinogenesis 1993; 14: 1737-1746.
24. Willett W. Nutritional epidemiology. Second edition. New York: Oxford University Press, 1998.

Chapter 2

Dietary fiber and plant foods in relation to colorectal cancer mortality: The Seven Countries Study

Margje CJF Jansen, H Bas Bueno-de-Mesquita, Ratko Buzina, Flaminio Fidanza, Alessandro Menotti, Henry Blackburn, Aulikki M Nissinen, Frans J Kok, Daan Kromhout

Int J Cancer 1999; 81: 174-179

Abstract

Many observational studies have found that higher consumption of vegetables, and to a lesser extent of fruits, was associated with lower risk of colorectal cancer. In particular, fiber or foods high in fiber have received attention in the potential prevention of colorectal cancer.

We performed an ecological analysis with data of the Seven Countries Study, to investigate whether intake of fiber and plant foods contributes to cross-cultural differences in 25-year colorectal cancer mortality in men. In the Seven Countries Study, around 1960 12,763 men aged 40 to 59 were enrolled in 16 cohorts in 7 countries. Baseline dietary information was gathered in small random samples per cohort, and nutrient intakes were based on chemical analyses of the average diets per cohort. Crude and energy-adjusted mortality rate ratios were calculated for a change of 10% of the mean intake of fiber and plant foods, i.e., total plant foods, fruits, vegetables, potatoes, grains, and related subgroups. Fiber intake was inversely associated with colorectal cancer mortality with an energy-adjusted rate ratio (RR) of 0.89 (95% confidence interval (CI) 0.80-0.97). An increase of 10 gram of daily intake of fiber was associated with a 33% lower 25-year colorectal cancer mortality risk. Intakes of vitamin B6 (RR 0.84, 95% CI 0.71-0.99) and α-tocopherol (RR 0.94, 95% CI 0.89-0.99) were also inversely associated with risk. Consumption of plant foods and related subgroups was not related to colorectal cancer.

It appears that fiber intake best indicates the part of plant food consumption, including whole grains, that is relevant for lowering colorectal cancer risk.

Introduction

Worldwide, cancer of the large bowel is the third most frequent type of cancer. Male colon cancer incidence rates vary 19-fold[1]. Migrant studies suggest that variance in incidence may be due largely to differences in dietary and other environmental factors, though genetic factors also play a role. There are few data to suggest that the dietary risk factors for rectal cancer are markedly different from those of colon cancer[2]. However, alcohol intake may be more involved in the etiology of rectal cancer.

It has been suggested repeatedly that consumption of vegetables and, to a lesser extent, of fruits is associated consistently with a lower risk of colorectal cancer. Among vegetable types, results for cruciferous vegetables are most consistent[3]. Fiber or foods high in fiber are inversely associated with colorectal cancer in most studies[2]. The protective effect of vegetables and fruits may be due to their many anticarcinogenic components, such as fiber, carotenoids, vitamins C and E, folate,

indoles, isothiocyanates and polyphenols. Whole grains contain many of the same compounds and, therefore, may share some of the beneficial properties of fruits and vegetables[4].

We conducted an ecological analysis, using the Seven Countries Study, to investigate whether consumption of fiber and plant foods, i.e., fruits, vegetables, potatoes and grains, and subgroups of these foods, could explain differences in 25-year colorectal cancer mortality in men. Moreover, intakes of plant-based dietary components, chemically analyzed in the average diet per cohort, were evaluated for their association. This ecological study is unique in that food consumption data were gathered in random sub-samples at the individual level, and cohort members were followed for 25 years.

Subjects and methods

Study design and participants

Between 1958 and 1964, 12,763 men aged 40-59 years from 16 cohorts were enrolled in the Seven Countries Study, with a participation rate of more than 90%[5]. Ten cohorts were established in rural areas of Finland (East and West), Italy (Crevalcore and Montegiorgio), Croatia (Dalmatia and Slavonia), Serbia (Velika Krsna), Greece (Crete and Corfu) and Japan (Tanushimaru); 2 cohorts of railroad employees in the United States and Italy (Rome Railroad); 1 of inhabitants of a small commercial market town in The Netherlands (Zutphen); 1 of workers in a large co-operative in Serbia (Zrenjanin); 1 of faculty members in Belgrade; and 1 fishing village in Japan (Ushibuka). Characteristics of these cohorts have been described elsewhere[6].

Dietary information

During the baseline survey, dietary information was collected in small random samples (8-49 men) of each of the 16 cohorts using the record method (Table 1). Diet was recorded for 7 days, except in Ushibuka (4 days), and in US Railroad (1 day). Collection took place between 1959 and 1964, with the exception of Rome Railroad and Ushibuka (around 1970). The dietary data were recoded in a standardized way by one dietitian in 1986[7]. However by then, the Greek records were no longer available, and had to be reconstructed from the results of Greek dietary surveys[8] and food balance sheets from Greece in 1961-1965.

Our main focus was on plant foods, i.e., fruits, vegetables, potatoes and grains. We considered plant foods as such (vegetable oils and alcoholic beverages not

included), and defined several subgroups, mainly based on botanical taxonomy and phytochemical content. Intakes of legumes, fruit preserves and fruit juices were included in consumption of plant foods and of fruits, respectively. Figure 1 presents an overview of the plant food groups investigated. The exact classification is available on request. Because of its relation with colorectal cancer, our analyses also included meat consumption, which was mainly red meat.

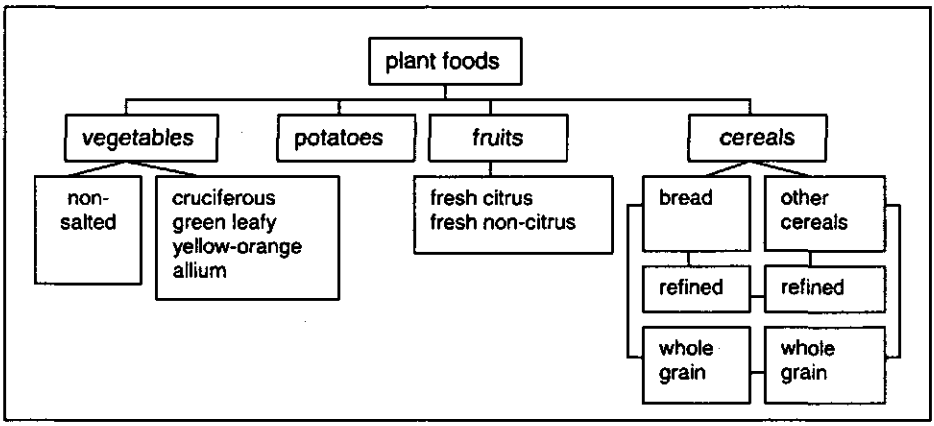


Figure 1 Plant food groups investigated for their relationship with 25-year colorectal cancer mortality in the Seven Countries Study

In addition to food groups, intakes of dietary components were investigated: dietary fiber, polygalacturonic acid (approximately 80% of pectin), β-carotene, vitamin C, vitamin B6, folate and α-tocopherol. In human diets, plant foods are the main source of fiber, polygalacturonic acid, β-carotene and vitamin C. Pectin is more present in fruits and vegetables than in grains. Both vegetable and animal foods contain vitamin B6, folate and α-tocopherol.

In 1987, food-equivalent composites representing the baseline average food intake in each of the 16 cohorts, were collected locally and chemically analyzed in a central laboratory according to a strict protocol. Chemical analyses for macronutrients, fiber, polygalacturonic acid and minerals were performed at the laboratory of the Department of Human Nutrition, Wageningen Agricultural University (Wageningen, The Netherlands). Vitamins were measured at the State Institute for Quality Control of Agricultural Products (Wageningen, The Netherlands). Methods of analysis were all according to standard methodology of that time and have been described elsewhere[9].

Mortality follow-up

During 25 years of follow-up, 5,974 men died. Data on vital status and causes of death were collected by means of death certificates, medical records, and interviews

of physicians and relatives of the deceased. Underlying cause of death was coded by two central reviewers according to the Eighth Revision of the International Classification of Diseases (ICD). Overall, only 56 men (0.4%) were lost to follow-up. The endpoint in the present study is colorectal cancer mortality (ICD 153-154).

Statistical analysis

Correlations presented are Pearson correlation coefficients; non-normal distributed variables have been log-transformed. Poisson regression was used to examine associations between dietary intake and colorectal cancer in terms of mortality rate ratios (PROC GENMOD, SAS statistics version 6.12). Two-sided *p*-values less than 0.05 were considered statistically significant. Number of colorectal cancer cases per cohort was the dependent variable. To adjust for differences in cohort size and survival time, the $\log_e$ of the person-time per cohort was used as the offset variable. Cohort mean daily intakes of fiber, plant foods and other dietary variables were the independent variables. Analyses were carried out for a change in intake of 10% of the mean overall consumption. Age, smoking, physical activity, BMI and intakes of energy, total fat, saturated fat, and alcohol were considered as potential confounders.

Baseline data on age, smoking, and physical activity were collected by a standardized questionnaire[5]. Physical activity was coded into four categories (bedridden, sedentary, moderately active, hard physical work) based on information on occupation and usual activities, including part-time jobs and notable non-occupational exercise. Height and weight were measured in a standardized way at baseline. Information on intakes of energy, total fat, saturated fat and alcohol was derived from the dietary assessment method described above. Mean scores per cohort were used in the analyses. For smoking, prevalence of smokers per cohort was used. Because of the very narrow age-range (Table 1), age was not included in multiple analyses; moreover, analyses showed no association with age (data not shown). Univariate analyses for smoking, BMI, total and saturated fat intake and alcohol intake did not indicate an association for any of these variables with colorectal cancer mortality (results not shown). For energy intake (RR 0.88, 95% CI 0.76-1.01) and physical activity (RR 0.90, 95% CI 0.79-1.02), inverse relationships with colorectal cancer mortality were indicated, although not statistically significant. Energy intake, considered most reliable of the two, and physical activity were statistically significant correlated among cohorts (r=0.52). Consequently, risk estimates were adjusted for intake of energy only. Further adjustment for smoking did not change the results, and is therefore not reported.

Results

Cohort size, mean age, number of men from whom dietary information was collected, and cancer mortality data of the 16 cohorts of the Seven Countries Study are shown in Table 1. During the 25-year follow-up period, 1580 men died of cancer, of whom 162 due to colorectal cancer (100 colon and 62 rectum). The highest age-adjusted colorectal cancer mortality, 2%, was observed in Zutphen and US Railroad, and the lowest, 0.1%, in East Finland.

Table 1 Cohort size, mean age, number of men from whom dietary information was gathered and 25-year cancer and colorectal cancer mortality of the 16 cohorts of the Seven Countries Study, 1958-1989

Cohort	Country	Number of men at baseline	Mean age (years)	Number dietary information	Cancer mortality[1] (%)	Colorectal cancer mortality N (%)[1]	Rate
US Railroad	USA	2571	49.8	30	11.1	50	2.0
East Finland	Finland	817	49.0	30	12.6	1	0.1
West Finland	Finland	860	50.1	30	12.3	8	0.9
Zutphen	Netherlands	878	50.1	45	17.7	17	2.0
Crevalcore	Italy	993	50.0	29	17.0	15	1.6
Montegiorgio	Italy	719	49.6	35	12.5	7	1.1
Rome Railroad	Italy	768	48.7	49	12.2	10	1.4
Dalmatia	Croatia	671	50.6	24	9.5	4	0.5
Slavonia	Croatia	696	50.5	24	10.4	8	1.1
Velika Krsna	Serbia	511	49.9	21	10.3	8	1.7
Zrenjanin	Serbia	516	49.2	40	13.1	8	1.4
Belgrade	Serbia	536	47.8	41	8.4	4	0.6
Crete	Greece	686	49.2	31	8.4	6	0.9
Corfu	Greece	529	49.8	37	10.7	2	0.4
Tanushimaru	Japan	508	50.7	24	12.6	9	1.6
Ushibuka	Japan	502	50.0	8	18.3	5	1.0

[1] N=number; both number and rate age-adjusted

Baseline representative daily intakes of fiber and some of the other components analyzed in the food composites, and mean daily consumption of plant foods, several plant food groups and meat are given in Table 2. Fiber intake was correlated with consumption of plant foods (r=0.69), potatoes (r=0.49), and whole grain bread (r=0.69).

Table 3 shows results of Poisson regression analyses for fiber, plant foods, some dietary components and meat. Fiber intake was inversely associated with colorectal cancer mortality (energy-adjusted RR 0.89, 95% CI 0.80-0.97). This means that an increase of 10 gram of daily intake of fiber was associated with a 33% lower

Table 2 Baseline representative daily intake of dietary fiber and other components analyzed in the food composites and baseline mean daily consumption of several plant food groups and meat in the 16 cohorts of the Seven Countries Study

Cohort	Dietary fiber	Poly-galacturonic acid[1]	Plant foods	Vegetables	Potatoes	Fruits	Grains	Meat	Vitamin B6	Folate	α-Tocopherol
	(g)								(mg)		
US Railroad	22.5	2.8	652	170	124	233	124	273	1.76	0.70	6.84
East Finland	50.0	3.7	874	108	273	40	451	105	2.33	1.02	9.56
West Finland	43.0	3.3	897	104	296	34	455	107	1.81	1.05	9.08
Zutphen	25.2	3.1	833	227	252	82	270	138	1.57	0.64	8.55
Crevalcore	25.3	2.9	864	140	30	191	498	154	1.66	0.89	15.71
Montegiorgio	27.6	2.6	827	194	56	28	528	85	1.66	0.64	13.16
Rome Railroad	26.5	2.2	807	260	29	150	362	224	2.03	0.90	14.32
Dalmatia	30.1	2.4	925	200	214	6	499	117	1.64	0.96	15.89
Slavonia	36.7	2.5	928	198	129	1	560	188	1.53	0.98	11.22
Velika Krsna	38.8	2.7	990	115	28	1	823	70	1.39	1.03	7.84
Zrenjanin	34.8	3.1	1090	245	136	185	509	212	1.96	0.75	12.11
Belgrade	25.6	3.4	715	179	86	145	293	175	1.57	0.53	18.27
Crete	43.1	2.7	1285	191	190	464	410	35	1.82	0.88	21.39
Corfu	57.2	3.6	1328	191	150	462	495	35	2.30	0.98	31.23
Tanushimaru	24.3	2.2	973	174	95	26	575	8	1.37	0.60	4.65
Ushibuka	21.0	1.4	888	222	34	42	511	8	1.51	0.72	6.30

[1] part of dietary fiber; polygalacturonic acid represents approximately 80% of the pectin complex (water-soluble fiber)

Table 3 Relations between baseline mean daily intake of several dietary components and food groups and mortality from colorectal cancer in the Seven Countries Study, 1958-1989

	10% of mean intake	Crude			Adjusted for energy intake		
		Rate ratio	95% CI	*p* value	Rate ratio	95% CI	*p* value
Dietary fiber	3.32 g	0.88	0.81-0.95	< 0.001	0.89	0.80-0.97	0.012
Polygalac-turonic acid	0.28 g	0.94	0.82-1.08	0.342	0.96	0.84-1.10	0.555
Plant foods	93.0 g	0.88	0.77-1.00	0.050	0.91	0.79-1.03	0.183
Grains	46.0 g	0.95	0.89-1.01	0.103	0.98	0.89-1.06	0.573
Bread	32.7 g	0.96	0.92-1.01	0.107	0.98	0.91-1.06	0.681
Whole grain bread	17.8 g	0.98	0.95-1.00	0.097	0.98	0.95-1.01	0.272
Vegetables	18.2 g	1.03	0.93-1.15	0.564	1.00	0.89-1.12	0.973
Fruits	13.1 g	1.00	0.98-1.02	0.923	0.99	0.96-1.02	0.514
Vitamin B6	0.17 mg	0.84	0.70-1.00	0.058	0.84	0.71-0.99	0.041
Folate	0.08 mg	0.89	0.79-1.01	0.063	0.93	0.78-1.10	0.391
α-Tocopherol	1.29 mg	0.93	0.88-0.98	0.011	0.94	0.89-0.99	0.040
Meat	12.1 g	1.04	1.00-1.07	0.022	1.03	1.00-1.06	0.094

colorectal cancer mortality risk. In Figure 2, fiber intake is plotted against the age-adjusted 25-year colorectal cancer mortality. Analyses for fiber with one cohort removed at a time indicated a robust relationship (data not shown). Analyses after removal of the two cohorts with the highest fiber intake, i.e., East-Finland and Corfu, resulted in an association of 0.93 (95% CI 0.85-1.00) and energy-adjusted 0.95 (95% CI 0.85-1.05). Polygalacturonic acid was not related to colorectal cancer mortality.

Consumption of plant foods was inversely related to colorectal cancer mortality approaching statistical significance (RR 0.88, 95% CI 0.77-1.00). Adjustment for energy intake attenuated this association (RR 0.91, 95% CI 0.79-1.03). Plant food groups showed no relationship with 25-year colorectal cancer mortality. Only crude analyses for grains (RR 0.95, 95% CI 0.89-1.01), bread (RR 0.96, 95% CI 0.92-1.01) and whole grain bread (RR 0.98, 95% CI 0.95-1.00) gave some indication of an inverse association.

We observed a statistically significant inverse association for intake of α-tocopherol (RR 0.93, 95% CI 0.88-0.98), whereas the intakes of folate (RR 0.89, 95% CI 0.79-1.01) and vitamin B6 (RR 0.84, 95% CI 0.70-1.00) were inversely related with borderline significance. Adjustment for energy intake did not materially

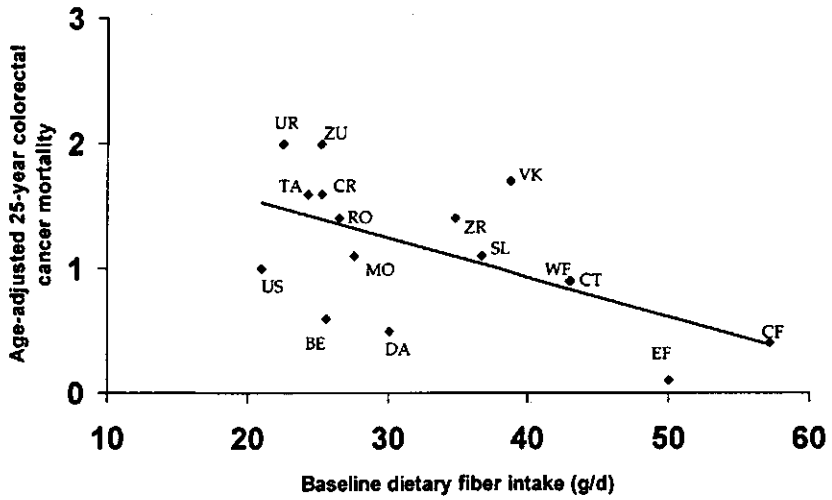


Figure 2 Association between baseline dietary fiber intake (g) and 25-year colorectal cancer mortality (%) in the Seven Countries Study, 1958-1989
UR, US Railroad; EF, East Finland; WF, West Finland; ZU, Zutphen; CR, Crevalcore; MO, Montegiorgio; RO, Rome Railroad; DA, Dalmatia; SL, Slavonia; VK, Velika Krsna; ZR, Zrenjanin; BE, Belgrade; CT, Crete; CF, Corfu; TA, Tanushimaru; US, Ushibuka

influence the risk estimates for α-tocopherol and vitamin B6, although the association with vitamin B6 reached statistical significance. The rate ratio for folate, however, was attenuated by adjustment for energy. Intakes of β-carotene and vitamin C were not related to risk.
Intake of fiber was highly correlated with intakes of vitamin B6 (r=0.66), folate (r=0.70) and to a lesser extent α-tocopherol (r=0.52). Due to multicollinearity, we could not examine the combined effect of these components.

Consumption of meat was positively associated with colorectal cancer mortality in crude analyses only (RR 1.04, 95% CI 1.00-1.07). Although this relationship did not hold after adjustment for energy intake, we evaluated the potential confounding effect of meat consumption. Adjustment for intake of energy and meat did not materially change the relationship between fiber and colorectal cancer (RR 0.90, 95% CI 0.81-0.99), further attenuated the association with plant foods (RR 0.97, 95% CI 0.80-1.16), and strengthened the inverse relation with vitamin B6 (RR 0.78, 95% CI 0.67-0.91).

Discussion

We studied the role of dietary fiber and plant foods in colorectal cancer mortality among men at the population level. Fiber intake was consistently related to reduced population risk: a 10-gram increase in daily fiber intake was associated with a 33% lower 25-year colorectal cancer mortality risk. Moreover, intakes of vitamin B6 and α-tocopherol were inversely associated with colorectal cancer mortality. Although consumption of fiber and plant foods was highly correlated, plant foods or subgroups were not related to risk. It seems that fiber intake best indicates the part of plant food consumption, including whole grains, that is relevant for lowering colorectal cancer risk.

Ecological analysis for testing etiological hypotheses has its limitations, mainly by the potential for substantial bias in effect estimation. The central problem is known as "ecological fallacy"[10]. The advantages of this design are a large variation in both exposure and outcome, and relatively small measurement errors in the exposure. In contrast to our study, most other correlation studies use per-capita-disappearance data to assess dietary intake, resulting in a poor estimate of intake; they do not investigate the same population for exposure and outcome; and they do not fulfill the temporality criterion. In addition, we chemically analyzed the nutrients studied. However, our study did have some disadvantages: 16 cohorts is a small number; dietary records were kept by only a subgroup of the cohort; 25 years passed between the dietary assessment method and the buying of the foods to be analyzed; food consumption around 1960 may not be an appropriate indicator for average food consumption during 25 years of follow-up. Regarding the latter, although differences in food consumption patterns have diminished, characteristic differences between the cohorts were still present after 20 years[7]. Furthermore, the assignment of statistical significance is complicated by the large number of models analyzed, making it possible that some associations have occurred by chance.

We are not aware of ecological studies relating plant food consumption with colorectal cancer. Studies on percentage vegetable calories[11] show a weak inverse correlation with colorectal cancer (r around -0.3). In our study and other cross-cultural studies, fruit and vegetable consumption was not related to colorectal cancer[12-14]. An ecological study in Britain, however, showed an inverse correlation for vegetables other than potatoes with death rates from colon cancer[15]. Our finding is in contrast with results from most case-control studies. Vegetable consumption, especially, is consistently inversely associated with risk[2]. Possibly this is due to the study design: associations at the individual level are not necessarily the same as those at the population level. This depends mainly on the distribution of other risk

factors within populations. Because the possibilities of controlling for other factors are limited, residual confounding may be present. Furthermore, the variation in vegetable intake between cohorts was moderate.

Grain consumption has been inversely associated with colorectal cancer in ecological studies[13,14,16]. In crude analyses, we found indications for an inverse association for grains, which appeared to be based mainly on whole grains. This result is in line with other findings, since all but one[13] presented crude relations only.

We consistently found an inverse association of colorectal cancer with chemically analyzed intake of fiber. For pectin, water-soluble fiber, no relation was found. In most correlation studies[13,17], but not all[15,18], inverse associations between fiber intake and colorectal cancer were seen. The study by Bingham *et al.*[15] compared regions of Britain and therefore had a smaller variation in fiber intake. Leaving out the two cohorts with the highest fiber intake weakened the risk estimates; however, non-significant inverse associations were still present. It seems that the association found for fiber was substantially driven by the cohorts with an average fiber intake of 50 grams per day or more. This may perhaps indicate that the amount of fiber necessary to produce a benefit may exceed 30 to 40 grams per day.

Many years ago, Burkitt[19] hypothesized that fiber may protect against cancer of the large bowel. He attributed the low incidence of bowel disease among Africans to the bulkiness of their stools, due to high consumption of whole plant foods, in particular a high intake of fiber. Possible mechanisms of action include reduction of transit time, binding of bile acids, fermentation to volatile fatty acids which may be directly anti-carcinogenic, and/or reducing the conversion of primary to secondary bile acids by lowering pH.

Since grains contain not only fiber, but also folate and vitamin B6, the association seen for fiber may be partly caused by those vitamins, or even other components. We are not aware of ecological studies investigating the association of vitamin B6 and folate with colorectal cancer. Experimental, case-control and cohort studies suggest possible involvement of folate in colon cancer etiology[20]: we therefore tested this relationship in our study. Although we found an indication for an association, no statistical significance was reached. Folate is involved in DNA synthesis and is an important methyl donor in methionine metabolism. Impaired DNA synthesis and deregulation in DNA methylation may enhance the risk of large bowel cancer. Both folate and vitamin B6 play a role in methionine metabolism and in the synthesis of nucleotide precursors of DNA[21]. In the present study, the association with vitamin B6 was strong and persisted after adjusting for intake of energy and meat.

For folate, it appeared that the microbiological assay used measured only the monoglutamates, i.e., the free form of folate. In foods, however, folates are mainly in the form of polyglutamates. The sum of mono- and polyglutamates is known as total folate. It has been suggested that free folate is more available to humans; however, since polyglutamates are broken down to monoglutamates in the intestine, total folate may be of main interest. We assume that the ranking of the cohorts according to intake of free folate corresponds with that of total folate intake. Yet this may not be true, since the ratio of free folate to total folate in foods is not constant, but depends on several conditions.

An inverse association was found between α-tocopherol intake and colorectal cancer mortality risk. Results on this relationship are not consistent in the literature. α-Tocopherol was also correlated with fiber intake. It is not clear whether fiber intake, the antioxidant capacity of α-tocopherol itself, or another mechanism, was responsible for the association.

In conclusion, high fiber intake was strongly associated with low colorectal cancer mortality, while intakes of vitamin B6 and α-tocopherol were also inversely related to risk at the population level. An increase of 10 gram in the daily intake of fiber was associated with a 33% lower risk of 25-year colorectal cancer mortality. Consumption of plant foods or plant food groups was not related to colorectal cancer. Dietary fiber appears to be an indicator for the part of plant food associated with low colorectal cancer risk in men at the population level.

Acknowledgments

The data for the present study were collected under the leadership of Dr. A Keys, Minneapolis, MN, USA, by Drs C Aravanis, AS Dontas, MJ Karvonen, I Mohacek, S Nedeljkovic and H Toshima (other principal investigators are co-authors). We are grateful to Dr. BPM Bloemberg, Dr. S Giampaoli, Ms. HE Goddijn, Ms. AM Jansen, Dr. R Schlemper and Dr. WMM Verschuren, for their contribution to the collection and preparation of the food-equivalent composites, to Mr. P van de Bovenkamp and Dr. MB Katan, Department of Human Nutrition and Epidemiology, Wageningen Agricultural University, and Dr. PCH Hollman, State Institute for Quality Control of Agricultural Products Wageningen, for preparation and chemical analyses of the food-equivalent composites.

References

1. Tomatis L, Aitio A, Day NE, Heseltine E, Kaldor J, Miller AB, Parkin DM, Riboli E. (Eds.).

Cancer: Causes, occurrence and control. IARC Scientific Publications No. 100. Lyon, France: IARC, 1990.
2. Potter JD. Nutrition and colorectal cancer. Cancer Causes Control 1996; 7: 127-146.
3. Steinmetz KA, Potter JD. Vegetables, fruit, and cancer. I. Epidemiology. Cancer Causes Control 1991; 2: 325-357.
4. Jacobs DR Jr., Slavin J, Marquart L. Whole grain intake and cancer: a review of the literature. Nutr Cancer 1995; 24: 221-229.
5. Keys A (Ed.). Seven Countries: a multivariate analysis of death and coronary heart disease. Cambridge: Harvard University Press, 1980: 1-381.
6. Keys A et al. Epidemiological studies related to coronary heart disease: characteristics of men aged 40-59 in seven countries. Acta Med Scand 1967; 460(Suppl): 1-392.
7. Kromhout D, Keys A, Aravanis C, Buzina R, Fidanza F, Giampaoli S, Jansen A, Menotti A, Nedeljkovic S, Pekkarinen M, Simic BA, Toshima H. Food consumption patterns in the 1960s in seven countries. Am J Clin Nutr 1989; 49: 889-894.
8. Keys A, Aravanis C, Sdrin H. The diets of middle-aged men in two rural areas of Greece. Voeding 1966; 27: 575-586.
9. Jansen AM, Kromhout D, Bloemberg BPM. Voedingsonderzoek in het kader van de Zeven Landen Studie, 1985-1988 (in Dutch). RIVM-report 528907001. Bilthoven, The Netherlands: RIVM, 1989.
10. Morgenstern H. Uses of ecologic analysis in epidemiologic research. Am J Publ Health 1982; 72: 1336-1344.
11. Howell MA. Diet as an etiological factor in the development of cancers of the colon and rectum. J Chron Dis 1975; 28: 67-80.
12. Knox EG. Foods and diseases. Br J Prev Soc Med 1977; 31: 71-80.
13. McKeown-Eyssen GE, Bright-See E. Dietary factors in colon cancer: international relationships. Nutr Cancer 1984; 6: 160-170.
14. Schrauzer GN. Cancer mortality correlation studies. II. Regional associations of mortalities with the consumption of foods and other commodities. Med Hypotheses 1976; 2: 39-49.
15. Bingham S, Williams DRR, Cole TJ, James WPT. Dietary fibre and regional large bowel cancer mortality in Britain. Br J Cancer 1979; 40: 456-463.
16. Armstrong B, Doll R. Environmental factors and cancer incidence and mortality in different countries, with special reference to dietary practices. Int J Cancer 1975; 15: 617-631.
17. McMichael AJ, Potter JD, Hetzel BS. Time trends in colorectal cancer mortality in relation to food and alcohol consumption: United States, United Kingdom, Australia and New Zealand. Int J Epidemiol 1979; 8: 295-303.
18. Drasar BS, Irving D. Environmental factors and cancer of the colon and breast. Br J Cancer 1973; 27: 167-172.
19. Burkitt WP. Epidemiology of cancer of the colon and rectum. Cancer 1971; 28: 3-13.
20. Glynn SA, Albanes D. Folate and cancer: a review of the literature. Nutr Cancer 1994; 22: 101-119.
21. Ma J, Stampfer MJ, Giovannucci E, Artigas C, Hunter DJ, Fuchs C, Willett WC, Selhub J, Hennekens C, Rozen R. Methylenetetrahydrofolate reductase polymorphism, dietary interactions, and risk of colorectal cancer. Cancer Res 1997; 57: 1098-1102.

Chapter 3

Consumption of plant foods and stomach cancer mortality in the Seven Countries Study. Is grain consumption a risk factor?

Margje CJF Jansen, H Bas Bueno-de-Mesquita, Leena Räsänen, Flaminio Fidanza, Alessandro Menotti, Aulikki M Nissinen, Edith JM Feskens, Frans J Kok, Daan Kromhout

Nutr Cancer 1999; 34: 49-55

Abstract

Plant foods are generally considered to be beneficial for health. A higher consumption of fruits, and to a lesser extent vegetables, is consistently associated with a lower risk of stomach cancer. Results on the association between stomach cancer and grain consumption are less clear.

We associated plant food consumption with 25-year stomach cancer mortality at population level in the Seven Countries Study. Around 1960, over 12,000 men aged 40-59 years from 7 countries and 16 cohorts were enrolled. In each cohort, dietary information was collected in small random samples. Crude and adjusted associations were calculated for a change of 10% of mean intake. Results differed for the plant foods studied: an inverse association was observed for fruits (adjusted rate ratio (RR) 0.96, 95% confidence interval (CI) 0.91-0.99), a positive relation for refined grains (adjusted RR 1.07, 95% CI 1.03-1.12), and no association for total plant foods, vegetables, whole grains, and potatoes. A high intake of refined grains was correlated with a low consumption of fruits.

In conclusion, high intake of refined grains may increase stomach cancer risk. However, since adjustment could only be limited in this study, high intake of refined grains may just reflect the deleterious effect of a diet low in fruits or other characteristics associated with low fruit consumption.

Introduction

Although rates of stomach cancer are declining, this cancer remains a major health problem throughout the world, inasmuch as it is second in rank of incidence and mortality among cancers. Stomach cancer has a distinct geographic variation with high rates in Japan and low rates in the United States[1], and is more incident among lower socioeconomic groups[2]. Diet and other environmental factors are assumed to be most important in the etiology of stomach cancer. High fruit and vegetable consumption is consistently associated with low risk. Consumption of grains and high-carbohydrate/starch diets are postulated to increase risk; however, results are not consistent[3]. Furthermore, intake of whole grains and dietary fiber is mostly inversely associated with risk[4,5]. Salt/salted foods and *N*-nitroso compounds are thought to increase risk, although findings vary[3]. Finally, smoking and *Helicobacter pylori* infection are risk factors for stomach cancer, whereas alcohol intake seems not related to risk in most studies[3].

In a cross-cultural analysis in the Seven Countries Study, an inverse association was observed between vitamin C and stomach cancer mortality[6]. Using the same

data and study design, we investigated the association at food group level for the consumption of plant foods, i.e., fruits, vegetables, potatoes, and grains, with 25-year stomach cancer.
The Seven Countries Study covers a wide range of food intake, and this ecological study is unique, in that food consumption data were gathered in random subsamples at the individual level and cohort members were followed for 25 years.

Subjects and methods

Study design and participants

The study design and participants of the Seven Countries Study have been described in detail elsewhere[7,8]. Briefly, 12,763 men aged 40-59 years in 16 cohorts from 7 countries were enrolled between 1958 and 1964. The number of men per cohort and the countries are mentioned in Table 1. Most cohorts were situated in rural areas, except for two railroad cohorts, the large agroindustrial cooperative Zrenjanin, faculty members of Belgrade University, Zutphen with inhabitants of a commercial market town, and the fishing village Ushibuka in Japan. The overall participation rate was over 90%.
Participants were followed for 25 years, and during that period 5,974 men died. Data on vital status and causes of death were collected by means of death certificates, medical records, and interviews of physicians and relatives of the deceased. Overall, only 56 men (0.4%) were lost to follow-up. Underlying cause of death was coded by two central reviewers[9] according to the Eighth Revision of the International Classification of Diseases (ICD). The endpoint in the present study is mortality from stomach cancer (ICD 151).

Dietary information

During the baseline survey, the record method was used to collect dietary information in small random samples (8-49 men) of each of the cohorts (Table 1). Diet was recorded for seven days, except in Ushibuka (4 days) and US Railroad (1 day) cohorts. Collection took place between 1959 and 1964, with the exception of Rome Railroad and Ushibuka cohorts (around 1970). The dietary data were recoded in a standardized way by one dietitian in 1986, and the average daily food intake per cohort was calculated[10]. However, by then, the Greek records were no longer available and had to be reconstructed by results of Greek dietary surveys[11] and food balance sheets from Greece in 1961-65. Furthermore, food equivalent composites representing the baseline average food intake in each of the 16 cohorts were collected locally and chemically analyzed in a central laboratory according to a strict protocol in 1987[12]. Chemical analyses for oligosaccharides, starch, and dietary fiber

were performed at the laboratory of the Department of Human Nutrition, Wageningen Agricultural University (Wageningen, The Netherlands). Vitamins were measured at the State Institute for Quality Control of Agricultural Products (Wageningen, The Netherlands). All methods of analysis were according to standard methodology of that time and have been described in detail elsewhere[13].

We investigated the role of total plant foods (vegetable oils and alcoholic beverages not included) and several plant food groups. Citrus and noncitrus fruits were examined separately. Grains were divided into whole grains and refined grains and as eaten in bread vs. other sources. Intakes of legumes, fruit preserves, and fruit juices were included in consumption of plant foods and fruits, respectively, but not investigated as separate groups.

Statistical analysis

Poisson regression was used to examine associations between dietary intake and stomach cancer mortality (PROC GENMOD, SAS statistics version 6.12)[14]. Number of stomach cancer cases per cohort was the dependent variable. To adjust for differences in cohort size and survival time, the $\log_e$ of the total survival time per cohort was used as the offset variable. Cohort mean daily intakes were the independent variables. Analyses were carried out for a change in intake of 10% of the mean consumption, both crude and adjusted for potential confounders. Because the number of cohorts was small (n=16), adjustments were made for only two variables at a time. On the basis of the literature and univariate analyses (data not shown), it was decided to adjust for smoking (prevalence) and energy intake (cohort mean daily intake). Alcohol consumption was not related to 25-year stomach cancer mortality (data not shown). Moreover, there was no adjustment for age, because the range in this study is very narrow (Table 1). Baseline data on smoking were collected by a standardized questionnaire[8]. Information on intakes of energy and alcohol was derived from the dietary record method described above.
Two-sided *p*-values less than 0.05 were considered statistically significant. Spearman correlation coefficients are presented.

Results

Baseline cohort size, mean age, smoking prevalence, energy intake, number of men from whom dietary information was collected, and 25-year total and stomach cancer mortality of the 16 cohorts of the Seven Countries Study are shown in Table 1. Stomach cancer mortality was highest in both Japanese cohorts (5.1%) and lowest in the Belgrade cohort (0.2%). Baseline mean daily consumption of plant

Table 1 Baseline cohort size, mean age, prevalence of smoking, energy intake, number of men from whom dietary information was gathered and 25-year total and stomach cancer mortality of the 16 cohorts of the Seven Countries Study, 1958-1989

Cohort	Country	Cohort size	Mean age (years)	Smokers (%)	Energy intake (MJ/d)	No. dietary information	Total mortality[1] (%)	Stomach cancer mortality	
								N	rate (%)[2]
US Railroad	USA	2,571	49.8	59.0	9.6	30	45.1	13	0.5
East Finland	Finland	817	49.0	68.5	14.7	30	59.7	23	2.9
West Finland	Finland	860	50.1	57.2	14.4	30	50.3	21	2.3
Zutphen	Netherlands	878	50.1	74.5	11.3	45	48.0	15	1.7
Crevalcore	Italy	993	50.0	62.6	15.2	29	49.8	32	3.2
Montegiorgio	Italy	719	49.6	58.7	12.1	35	46.2	29	4.0
Rome Railroad	Italy	768	48.7	65.2	11.0	49	39.7	10	1.6
Dalmatia	Croatia	671	50.6	58.3	15.8	24	43.3	7	1.0
Slavonia	Croatia	696	50.5	60.3	15.5	24	61.0	27	3.8
Velika Krsna	Serbia	511	49.9	49.2	14.3	21	50.0	14	2.6
Zrenjanin	Serbia	516	49.2	63.2	13.4	40	57.9	10	1.8
Belgrade	Serbia	536	47.8	43.7	11.4	41	29.5	2	0.2
Crete	Greece	686	49.2	57.3	11.8	31	31.4	3	0.5
Corfu	Greece	529	49.8	63.5	11.3	37	40.4	7	1.3
Tanushimaru	Japan	508	50.7	70.7	10.0	24	39.4	28	5.1
Ushibuka	Japan	502	50.0	77.8	10.2	8	51.5	26	5.1

[1] age-adjusted; [2] N = number, both number and rate are age-adjusted

Table 2 Baseline mean daily consumption in grams of several plant food groups and baseline representative daily intake in grams of several components analysed in the food composites in the 16 cohorts of the Seven Countries Study

Cohort	Plant foods	Fruits	Vegetables	Grains	Whole grains	Refined grains	Potatoes	Carbohydrates	Starch
					(g)				
US Railroad	652	233	170	124	24	100	124	182	63
East Finland	874	40	108	451	288	163	273	411	194
West Finland	897	34	104	455	268	187	296	453	245
Zutphen	833	82	227	270	64	206	252	293	126
Crevalcore	864	191	140	498	0	498	30	384	207
Montegiorgio	827	28	194	528	0	528	56	355	235
Rome Railroad	807	150	260	362	8	354	29	258	147
Dalmatia	925	6	200	499	202	298	214	361	227
Slavonia	928	1	198	560	76	485	129	401	236
Velika Krsna	990	1	115	823	686	137	28	462	369
Zrenjanin	1,090	185	245	509	399	110	136	358	207
Belgrade	715	145	179	293	131	162	86	272	123
Crete	1,285	464	191	410	380	30	190	323	223
Corfu	1,328	462	191	495	450	45	150	331	221
Tanushimaru	973	26	174	575	0	575	95	442	334
Ushibuka	888	42	222	511	0	511	34	404	315

foods and several components as measured in the food composites are given in Table 2. The Seven Countries Study covers a wide range on intake of plant foods, especially for fruits and grains, and to a lesser extent for vegetables (Table 2).

Table 3 shows results, crude and adjusted for energy intake and smoking, of the Poisson regression analyses for consumption of plant foods, vitamin C, carbohydrates, starch, oligosaccharides, and dietary fiber. Total plant food consumption was not related to stomach cancer risk, whereas the intake of fruits was inversely associated to risk. After removal the two cohorts with the highest fruit intake, i.e., Crete and Corfu, the inverse association found for fruits was still statistically significant (data not shown). Our results indicated that citrus fruits were most responsible for the inverse relation with fruits. However, one-half of the cohorts consumed no or a very low amount of citrus fruits per day. Six cohorts ate less than 2 grams, whereas three cohorts consumed no citrus fruits. Adjustment for energy intake and prevalence of smoking did not materially change the results. Intake of vegetables was not related to risk, whereas the adjusted association found for potatoes was of borderline significance.

Table 3 Relations for 25-year mortality from stomach cancer in men in the Seven Countries Study with baseline mean daily consumption of food groups, and baseline representative daily intake of components analyzed in food composites. Poisson regression for a change of 10% of the mean intake

	10% of mean intake	Crude		Adjusted for energy and smoking	
		Rate ratio	95% CI	Rate ratio	95% CI
Plant foods	93.0 g	1.06	0.87-1.27	1.02	0.81-1.24
Fruits and vegetables	31.3 g	0.89	0.82-0.96	0.90	0.82-0.98
Fruits	13.1 g	0.94	0.90-0.98	0.96	0.91-0.99
Citrus fruits	1.8 g	0.95	0.93-0.98	0.95	0.92-0.98
Non-citrus fruits	10.1 g	0.97	0.92-1.01	0.98	0.94-1.01
Vegetables	18.2 g	0.96	0.81-1.13	0.96	0.82-1.11
Grains	46.0 g	1.15	1.06-1.24	1.18	1.09-1.27
Whole grains	18.6 g	0.99	0.94-1.03	0.99	0.95-1.03
Refined grains	27.4 g	1.09	1.05-1.13	1.07	1.03-1.12
bread	14.8 g	1.03	0.98-1.07	1.01	0.97-1.06
other sources	12.6 g	1.03	1.01-1.05	1.04	1.02-1.06
Potatoes	13.3 g	0.98	0.91-1.04	0.96	0.92-1.00
Vitamin C	7.4 mg	0.89	0.84-0.94	0.89	0.85-0.93
Carbohydrates	35.6 g	1.30	1.16-1.47	1.30	1.15-1.47
Starch	21.7 g	1.15	1.07-1.24	1.14	1.07-1.22
Oligosaccharides	11.1 g	1.00	0.88-1.12	0.92	0.83-1.02
Dietary fiber	3.3 g	1.01	0.88-1.14	0.97	0.85-1.09

Grain consumption was positively related to stomach cancer risk. Separating this consumption into whole grains and refined grains revealed only a positive association for the latter group and, in particular, for grains from sources other than bread, e.g., rice and pasta.

At the nutrient level, carbohydrates and starch were positively associated with stomach cancer mortality, whereas oligosaccharides and dietary fiber were not related to risk. As reported elsewhere[8], vitamin C intake was inversely associated to risk (Table 3).

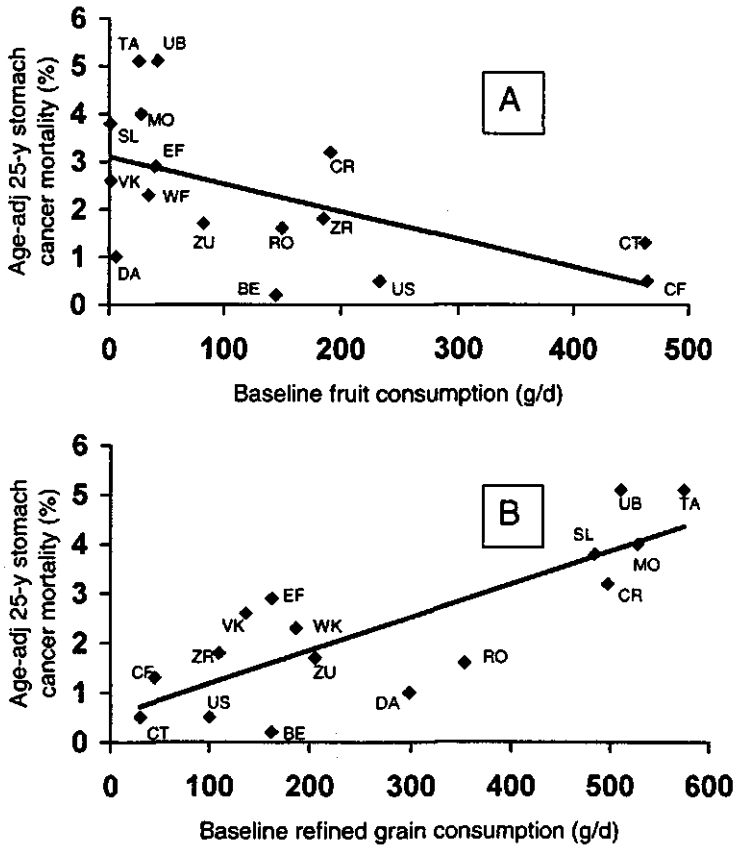


Figure 1 Association between baseline fruit consumption (g/d) (A) and refined grain consumption (g/d) (B) and age-adjusted 25-year stomach cancer mortality (%) in the Seven Countries Study, 1958-1989; US, US Railroad; EF, East Finland; WF, West Finland; ZU, Zutphen; CR, Crevalcore; MO, Montegiorgio; RO, Rome Railroad; DA, Daltmatia; SL, Slavonia; VK, Velika Krsna; ZR, Zrenjanin; BE, Belgrade; CT, Crete; CF, Corfu; TA, Tanushimaru; UB, Ushibuka

The inverse association of fruit consumption with age-adjusted 25-year stomach cancer mortality is shown in Figure 1A. Some cohorts ate almost no fruits (Slavonia and Velika Krsna), and others had a very high consumption (Crete and Corfu). Figure 1B depicts the positive relation between intake of refined grains and stomach cancer mortality. Compared with fruit intake, refined grain consumption has a wider range, and is better distributed along the range. Cohorts with a high fruit consumption ate little refined grains; the same is true in the opposite direction. Investigating whether associations for fruits and refined grains were independent was not considered feasible, because both intakes were highly correlated (r= -0.56, p <0.01).

Discussion

Cross-cultural analyses in the Seven Countries Study relating 25-year stomach cancer mortality with plant food consumption revealed an inverse association with fruits, a positive relation with refined grains, and no association with total plant foods, vegetables, whole grains, and potatoes.

Ecological analysis for testing etiological hypotheses has its limitations, mainly by the potential for substantial bias in effect estimation. The central problem is known as "ecological fallacy"[15]. Associations at the population level are not necessarily the same as those at the individual level. This depends mainly on the distribution of other risk factors within populations. The advantages of this design are a large variation in exposure and outcome and relatively small measurement errors in the exposure. Furthermore, in our study, dietary intake was assessed in small random samples, and cohort members were followed for 25 years. Yet, our study did have some disadvantages: 16 cohorts is a small number; dietary records were kept only by a subgroup of the cohort; food consumption around 1960 may not be an appropriate indicator for average food consumption during 25 years of follow-up. Regarding the last disadvantage, although differences in food consumption patterns have diminished, characteristic differences between the cohorts were still present after 20 years[10].

In our study, smoking and energy intake seemed to be the most important variables for which to adjust. We had very limited information on intake of salt and salted foods. Therefore, we decided not to adjust risk estimates on plant foods for these intakes. Furthermore, univariate analyses between salt intake and stomach cancer mortality did not show an association. *Helicobacter pylori* infection is positively associated with stomach cancer in most studies, and infection rates vary

throughout the world. Information on this factor, however, was lacking, and we could not evaluate its potential confounding effect.
We calculated risk estimates for a difference of 10% of the mean intake, because such a change is assumed to be a feasible and realistic goal in public health. For evaluating the magnitude of the associations, it should be noticed that this contrast is small compared with differences generally used in etiological studies.

Although generally considered a consistent finding, most cross-cultural correlation studies did not observe an inverse association between stomach cancer and fruit intake[16-20]. Using per capita disappearance data, not using an appropriate lag time, and the fact that exposure and outcome did not apply to the same population may have influenced these results. We did find an inverse relationship for fruits, perhaps because of the features of our ecological study. Correlation studies within countries and case-control and cohort studies did consistently find inverse associations for fruits with stomach cancer[3]. Fruits are the richest source of vitamin C in the diet. However, other foods, e.g., potatoes, can contribute substantially to intake. Vitamin C may protect against stomach cancer by inhibiting the formation of carcinogenic *N*-nitroso compounds[3]. Furthermore, fruits contain bioactive compounds other than vitamin C that may be responsible, separately or in combination, for the protective effect observed.

Vegetable intake was not associated with stomach cancer mortality in our study. Also other correlation studies did not report an association[16-20]. We found a smaller variance in intake for vegetables than for fruits. This may explain why fruits were inversely associated, whereas vegetables were not. Moreover, case-control studies on stomach cancer showed a more pronounced inverse association for fruits than for vegetables[21].

Epidemiological studies investigated the relationship of grains, high-carbohydrate/starch diets, whole grains, rice, pasta, bread, and components such as carbohydrates, starch, and dietary fiber with stomach cancer. Associations observed for these dietary items are not consistent. In line with our study, cross-cultural correlation studies found a positive association between grain consumption and stomach cancer mortality[17-19,22,23]. A Japanese correlation study also observed a positive relation[24], whereas in Spain no correlation was found[25]. Ecological findings on bread[17,25,26], rice[20,25-29], and pasta[25,26] were inconsistent. A review of case-control studies on consumption of grains[30] suggested an increased stomach cancer risk: in seven of eight studies, of which five were statistically significant[31-35], risk was found to increase. Some recent case-control studies on grain consumption[36,37] did not show an association with stomach cancer. For rice and pasta, variable results from

case-control studies were reported[30]. Case-control studies on bread revealed no association, and whole grain bread may decrease stomach cancer risk[4,5]. Only a few cohort studies on grain consumption have been published, and moderate, mostly nonsignificant, increased risks or no associations were reported[30].

Several mechanisms have been proposed for how grains or a high-carbohydrate/starch diet can increase stomach cancer risk. First, there may be physical irritation of the gastric mucosa. Second, such diets are generally low in proteins, leading to a reduced gastric mucus production facilitating carcinogen absorption[38]. However, protein intake has never been related to a decreased risk in case-control or cohort studies[30], and was not associated in the present study (data not shown). Another possibility hypothesized is that diets high in starch are also high in salt and that salt is the risk factor in such a diet[39]. Furthermore, it is suggested that a high-starch diet is generally a monotonous diet, low in fruits, vegetables, and whole grains; such a diet is low in bioactive components thought to be anticarcinogenic[30].

We observed a positive association for grains that could be attributed to refined grains. No association for refined bread intake was observed, whereas consumption of refined grains from sources other than bread was associated with an increased stomach cancer risk. At the nutrient level, we found the strongest positive association for intake of carbohydrates. When divided into starch, oligosaccharides, and dietary fiber, only an association for starch was present.

It seems that the suggestion of increased risk with grain consumption is mainly based on case-control studies on broad groups of grains. However, not all these studies adjusted for all relevant potential confounders. For instance, studies did not control for intake of fruits and/or vegetables[31,34], for total energy intake[31,32], or for smoking and socioeconomic status[34]. Socioeconomic status is correlated with several dietary and other lifestyle factors that are associated with stomach cancer itself[40], e.g., monotonous diets[2] and use of refrigerators[30]. In one study[35], a statistically significant positive trend was observed for grain consumption; however, this trend was no longer significant after further adjustment for education, smoking, energy, and vitamin C.

Given that not all these studies adjusted the risk estimates appropriately, the epidemiological evidence supporting the idea that grain consumption is a risk factor for stomach cancer is not as firm as sometimes stated. Furthermore, because associations differ for grain items studied, results for broad groups of grains may not be informative enough. This may have implications for the choice and/or design of dietary assessment methods.

Although we emphasized the need of appropriate adjustment, our ability to adjust our own study was limited. Fruit consumption was highly correlated with intake of refined grains in our study. Because of the small number of cohorts and the fact that adjustment for confounders can only be done crudely in this ecological study design, we could not examine whether both associations were independent. However, taking the evidence together, we hypothesize that the consumption of refined grains did not elevate the risk, but a high intake of these grains was an indicator for a diet low in fruits. Such a diet provides a low amount of bioactive compounds thought to protect against cancer. Furthermore, other nonmeasured factors correlated with a refined grain intake could be responsible for the observed association.
In case-control and cohort studies, multicollinearity is expected to be a smaller problem, so that after adequate adjustment, the independent effect of dietary factors can be evaluated. These types of studies may further elucidate whether grain consumption itself can be seen as a risk factor for stomach cancer.

Briefly, on the basis on our study, we cannot rule out the possibility that refined grain consumption is a risk factor for stomach cancer. However, we hypothesize that the consumption of refined grains did not elevate the risk, but a high intake of these grains was an indicator for a diet poor in fruits. Such a diet is low in bioactive compounds that may exert an anticarcinogenic effect on the development of stomach cancer.

Acknowledgments

We thank Dr A Keys for his drive and assiduity in carrying through the study for more than 25 years. We also thank the principal investigators of the Seven Countries Study not being co-authors: Drs C Aravanis, H Blackburn, R Buzina, AS Dontas, MJ Karvonen, I Mohacek, S Nedeljkovic, and H Toshima. We are grateful to Dr BPM Bloemberg, Dr S Giampaoli, HE Goddijn, Dr R Schlemper and Dr WMM Verschuren for their contribution to the collection and preparation of the food equivalent composites, to P van den Bovenkamp and Dr MB Katan, Dept of Human Nutrition and Epidemiology, Wageningen Agricultural University, and Dr PCH Hollman, State Institute for Quality Control of Agricultural Products, Wageningen for preparation and chemical analyses of the food equivalent composites. Dr DR Jacobs Jr. is thanked for his helpful comments on the manuscript.

References

1. Coleman MP, Estève J, Damiecki P, Arslan A, Renard H. Trends in Cancer Incidence and Mortality. IARC Sci Pub No 121. Lyon, France: IARC, 1993: 193-224.
2. Neugut AI, Hayek M, Howe G. Epidemiology of gastric cancer. Semin Oncol 1996; 23:

281-291.
3. Kono S, Hirohata T. Nutrition and stomach cancer. Cancer Causes Control 1996; 7: 41-55.
4. Jacobs DR Jr., Slavin J, Marquart L. Whole grain intake and cancer: A review of the literature. Nutr Cancer 1995; 24: 221-229.
5. Jacobs DR Jr., Marquart L, Slavin J, Kushi LH. Whole-grain intake and cancer: An expanded review and meta-analysis. Nutr Cancer 1998; 30: 85-96.
6. Ocké MC, Kromhout D, Menotti A, Aravanis C, Blackburn H, et al. Average intake of antioxidant (pro)vitamins and subsequent cancer mortality in the 16 cohorts of the Seven Countries Study. Int J Cancer 1995; 61: 480-484.
7. Keys A, Aravanis C, Blackburn HW, van Buchem FSP, Buzina R, et al. Epidemiological studies related to coronary heart disease: characteristics of men aged 40-59 in seven countries. Acta Med Scand 1967; 460(Suppl): 1-392.
8. Keys A (Ed.). Seven Countries: A multivariate analysis of death and coronary heart disease. Cambridge, Mass: Harvard University Press, 1980: 1-381.
9. Keys A, Menotti A, Karvonen MJ, Aravanis C, Blackburn HW, et al. The diet and 15-year death rate in the Seven Countries Study. Am J Epidem 1986; 124: 903-915.
10. Kromhout D, Keys A, Aravanis C, Buzina R, Fidanza F, et al. Food consumption patterns in the 1960s in seven countries. Am J Clin Nutr 1989; 49: 889-894.
11. Keys A, Aravanis C, Sdrin H. The diets of middle-aged men in two rural areas of Greece. Voeding 1966; 27: 575-586.
12. Vries de JHM, Jansen AM, Kromhout D, Bovenkamp van de P, Staveren van WA, et al. The fatty acid and sterol content of food composites of middle-aged men in seven countries. J Food Comp Anal 1997; 10: 115-141.
13. Jansen AM, Kromhout D, Bloemberg BPM. Voedingsonderzoek in het kader van de Zeven Landen Studie, 1985-1988 (in Dutch). RIVM-report 528907001. Bilthoven, The Netherlands: RIVM, 1989.
14. SAS Institue Inc. SAS technical report P-243. The GENMOD procedure, release 6.09. Cary, NC: SAS Institute Inc, 1993.
15. Morgenstern H. Uses of ecologic analysis in epidemiologic research. Am J Public Health 1982; 72: 1336-1344.
16. Howell MA. Factor analysis of international cancer mortality data and per capita food consumption. Br J Cancer 1974; 29: 328-336.
17. Schrauzer GN. Cancer mortality correlation studies. II. Regional associations of mortalities with the consumption of foods and other commodities. Med Hypotheses 1976; 2: 39-49.
18. Maruchi N, Aoki S, Tsuda K, Tanaka Y, Toyokawa H. Relation of food consumption to cancer mortality in Japan, with special reference to international figures. Jpn J Cancer Res 1977; 68: 1-13.
19. Tominaga S, Ogawa H, Kuroishi T. Usefulness of correlation analyses in the epidemiology of stomach cancer. Natl Cancer Inst Monogr 1982; 62: 135-140.
20. Knox EG. Foods and diseases. Br J Prev Soc Med 1977; 31: 71-80.
21. Steinmetz KA, Potter JD. Vegetables, fruit, and cancer. I. Epidemiology. Cancer Causes Control 1991; 2: 325-357.
22. Hakama M, Saxén EA. Cereal consumption and gastric cancer. Int J Cancer 1967; 2:

265-268.
23. Armstrong B, Doll R. Environmental factors and cancer incidence and mortality in different countries, with special reference to dietary practices. Int J Cancer 1975; 15: 617-631.
24. Kato I, Tominaga S, Kuroishi T. Per capita foods/nutrients intake and mortality from gastrointestinal cancers in Japan. Jpn J Cancer Res 1987; 78: 453-459.
25. Corella D, Cortina P, Guillén M, González JI. Dietary habits and geographic variation in stomach cancer mortality in Spain. Eur J Cancer Prev 1996; 5: 249-257.
26. Decarli A, LaVecchia C. Environmental factors and cancer mortality in Italy: correlational exercise. Oncology 1986; 43: 116-126.
27. Tsubono, Y, Kobayashi, M, and Tsugane, S: Food consumption and gastric cancer mortality in five regions of Japan. Nutr Cancer 1997; 27: 60-64.
28. Kneller RW, Guo W-D, Hsing AW, Chen J-S, Blot WJ et al. Risk factors for stomach cancer in sixty-five Chinese counties. Cancer Epidemiol Biomarkers Prev 1992; 1: 113-118.
29. Hirayama T. Epidemiology of cancer of the stomach with special reference to its recent decrease in Japan. Cancer Res 1975; 35: 3460-3463.
30. World Cancer Research Fund/American Institute for Cancer Research. Food, nutrition and the prevention of cancer: a global perspective. Washington, DC: American Institute for Cancer Research, 1997.
31. Modan B, Lubin F, Barell V, Greenberg RA, Modan M et al. The role of starches in the etiology of gastric cancer. Cancer 1974; 34: 2087-2092.
32. Risch HA, Jain M, Choi NW, Fodor JG, Pfeiffer CJ et al. Dietary factors and the incidence of cancer of the stomach. Am J Epidemiol 1985; 122: 947-959.
33. Trichopoulos D, Ouranos G, Day NE, Tzonou A, Manousos O et al. Diet and cancer of the stomach: a case-control study in Greece. Int J Cancer 1985; 36: 291-297.
34. Tuyns AJ, Kaaks R, Haelterman M, Riboli E. Diet and gastric cancer. A case-control study in Belgium. Int J Cancer 1992; 51: 1-6.
35. Ramón JM, Serra L, Cerdó C, Oromí J. Dietary factors and gastric cancer risk. Cancer 1993; 71: 1731-1735.
36. Cornée J, Pobel D, Riboli E, Guyader M, Hémon B. A case-control study of gastric cancer and nutritional factors in Marseille, France. Eur J Epidemiol 1995; 11: 55-65.
37. Harrison LE, Zhang Z-F, Karpeh MS, Sun M, Kurtz RC. The role of dietary factors in the intestinal and diffuse histologic subtypes of gastric adenocarcinoma. Cancer 1997; 80: 1021-1028.
38. Nomura A. Stomach cancer. In: Cancer epidemiology and prevention. Schottenfeld D, JF Fraumeni (Eds.). New York/Oxford: Oxford University Press, 1996.
39. Joossens JV, Hill JJ, Elliott P, Stamler R, Lesaffre E, et al. Dietary salt, nitrate and stomach cancer mortality in 24 counties. Int J Epidemiol 1996; 25: 494-504.
40. Howson CP, Hiyama T, Wynder EL. The decline in gastric cancer: epidemiology of an unplanned triumph. Epidemiol Rev 1986; 8: 1-27.

Chapter 4

Cohort analysis of fruit and vegetable consumption and lung cancer mortality in European men

Margje CJF Jansen, H Bas Bueno-de-Mesquita, Leena Räsänen, Flaminio Fidanza, Aulikki M Nissinen, Alessandro Menotti, Frans J Kok, Daan Kromhout

Int J Cancer 2001; 92: 913-918

Abstract

Our aim was to examine the relationship between fruit and vegetable consumption and lung cancer mortality in a cohort of European males. Around 1970, dietary intake of Finnish, Italian and Dutch middle-aged men was assessed using a cross-check dietary history. Complete baseline information was available for 3,108 men, of whom 1,578 baseline smokers. We used Cox proportional hazard analyses to calculate risk estimates for the consumption in country-specific tertiles on lung cancer in smokers. During 25 years of follow-up, 149 lung cancer deaths occurred in the smokers. Fruit consumption was inversely associated with lung cancer mortality among smokers; compared with the lowest, adjusted relative risks (RR) (95% confidence interval (CI)) for the intermediate and highest tertiles were 0.56 (0.37-0.84) and 0.69 (0.46-1.02), *p* trend 0.05. Only in the Dutch cohort was this association statistically significant (adjusted RRs (95% CI) 1.00; 0.33 (0.16-0.70); 0.35 (0.16-0.74), *p* trend 0.004). In Finland lung cancer risk was lower with higher fruit intake but not significantly, whereas in Italy no association was observed. Stratifying on cigarette smoking intensity (non, light and heavy) revealed an inverse association in the heavy smokers only (adjusted RRs (95% CI) 1; 0.47 (0.26-0.84); 0.40 (0.20-0.78)). Vegetable consumption was not related to lung cancer risk in smokers. However, analyses stratified on cigarette smoking intensity gave some indication for a lower lung cancer risk with higher intake.
In conclusion, in this prospective analysis among European smoking men, fruit intake was inversely related to lung cancer mortality. This association was confined to heavy cigarette smokers.

Introduction

Lung cancer is a major public health problem, because of both high incidence[1] and high fatality rates[2]. Cigarette smoking is the dominant risk factor for this type of cancer; with an attributable risk of 90 percent in men[3]. Additionally, diet may influence lung cancer risk. Many epidemiological studies have shown that fruit and vegetable intake is associated with a lower lung cancer risk[4-8]. The estimated relative risks for high vs. low consumption vary considerably between studies, and the overall estimate ranges from 0.45[5] to 0.5-0.7[8]; a formal meta-analysis has not been performed.
Although the inverse association between fruit and vegetable intake and lung cancer seems convincing, some issues have been raised that may weaken this relationship. First, residual confounding by smoking: Smoking is such a dominant factor in lung cancer etiology that adjustment for this risk factor, especially when only smoking status is used, may not fully remove its effect. Smokers tend to eat less fruit and vegetables[9], and probably have a less healthy lifestyle in general, which may result in

residual confounding. Second, it has been suggested that results from cohort studies, compared with case-control studies, are less conclusive[10]. Case-control studies may overestimate the association due to recall and selection bias.

Cigarette smokers have a high exposure to carcinogens. Therefore, potential anticarcinogens provided by fruit and vegetables are expected to exert their action especially in this group. However, results by smoking status are not consistent. Some studies found inverse associations especially in current smokers[11], in heavy smokers[12,13] and others in light smokers[14,15], in former smokers[14,16] or in nonsmokers[17,18].

We examined the relationship between fruit and vegetable consumption and lung cancer mortality in a prospective study among males (n=3,108), from Finland, Italy and The Netherlands, all participating in the Seven Countries Study. Baseline data from around 1970 were used and mortality was followed for 25 years. Because lung cancer mainly occurs in smokers, we restricted most analyses to baseline smokers (n=1,578).

Material and methods

Study population

Between 1958 and 1964, 16 population samples of men aged 40-59 years from seven countries were enrolled and examined for the Seven Countries Study, with a participation rate of more than 90%[19]. In Finland this study started in 1959, and in Italy and The Netherlands in 1960. Dietary intake was only assessed in a small subgroup per cohort at that time. Around 1965, a second round of this study was conducted, however, no dietary information was gathered in Finland. As baseline for the present analysis, we used the third round of the study, which was around 1970. We used data of five of the original 16 population samples: two in Finland (East Finland and West Finland), two in Italy (Crevalcore and Montegiorgio), and one in The Netherlands (Zutphen) because dietary data were available. The Dutch cohort consisted of inhabitants of a small commercial town, Zutphen, whereas the other cohorts were situated in rural areas. Information was gathered in Finland in 1969 (n=612 in East Finland and n=694 in West Finland), in Zutphen (n=615) and in Crevalcore (n=592) in 1970. In the Montegiorgio cohort, dietary information was gathered in 1970 only from a subset. Therefore, the dietary data collected in 1965 from the men still alive in 1970 were used as an approximation of dietary intake in 1970 (n=662 men). Although fruit and vegetable consumption was higher in the subset of men in 1970, differences were not statistically significant[20]. For analyses, complete information was available for 3,108 men, of whom 1,578 were cigarette smokers.

Examinations

Food intake around 1970 was estimated by using the cross-check dietary history method. This method provides information about the usual food consumption pattern six to twelve months preceding the interview[21]. First, the usual food consumption pattern of a person during weekdays and weekends was assessed. This part concerned questions about the foods used at breakfast, lunch, dinner and between the meals. Thereafter, a checklist with an extensive number of foods was used to calculate and to verify the participant's food consumption pattern. Experienced dietitians and nutritionists carried out the interviews. In Finland, the dietary surveys were held in autumn, in Italy and The Netherlands in spring. Although this method was adjusted to the local situation, the methodology was comparable. In all countries food items were categorized into food groups, such as fruit and vegetables, in the same way. However, the types of fruits and vegetables within the food groups could differ per country. The nutrient intake was assessed using computerized versions of the local food tables for the countries[22-24].

In all cohorts, information on age and smoking of the participants was collected in a standardized way[25]. Men were asked to report their current smoking status (never, former or current smoker). Current and former smokers reported the number of cigarettes they smoked or used to smoke per day (1-4, 5-9, 10-19, 20-29 or more than 30 cigarettes). The midpoints of these categories (2, 7, 15, 25 and 35) were used as number of cigarettes smoked per day. For 70 men, of which 63 from Montegiorgio, baseline information on smoking was missing. For 63 of the 70 men, smoking data of 1965 were appropriate to use as a proxy for the 1970 data.

No ethical guidelines existed at the time of the first surveys, however, the study was retrospectively approved by the Medical Ethical Committee of the University of Leiden, The Netherlands in 1985.

Follow-up

The participating men were followed for mortality during 25 years, i.e., from around 1970 to 1995. None of them was lost to follow-up. The underlying causes of death were coded in a standardized way by one reviewer, using the 8th revision of the WHO International Classification of Diseases (ICD). The cause of death was based on information from the official death certificate, in combination with information from medical and hospital records. In case of multiple causes of death, priority was given to accidents, followed by cancer in advanced stages, coronary heart disease and stroke. For the present analyses, lung cancer mortality was defined as ICD8 code 162 as the primary (n=183) or secondary (n=4) cause of death.

Statistical methods

Cox proportional hazard analysis was used to associate intake of fruit and vegetables with 25-year lung cancer mortality. Fruit intake was analyzed as total fruit consumption, that is the sum of fresh fruit, dried fruit multiplied by two (to account for water loss), canned fruit and fruit juices. In Italy and The Netherlands, almost all fruit was eaten as fresh fruit. In Finland, however, other forms of fruit, especially fruit juices (mainly berry juice), were substantially consumed (Table 1). Therefore, analyses for fresh fruit were performed as well. Vegetable consumption did not include potatoes. Consumption was divided in country-specific tertiles. Relative risks (RRs) and 95% confidence intervals (95% CI) were calculated, with the lowest tertile as the reference category. Statistical significance was determined by two-sided tests with a critical value of 0.05 or less. Countries were analyzed separately, combining the two cohorts in Finland and the two cohorts in Italy. Because no effect modification by country was present, analyses were also performed for all countries pooled, using the STRATA option of the PHREG procedure[26]. Survival analyses were done crudely, adjusted for age and number of cigarettes per day (model 1), and for model 1 plus energy intake, fruit intake (in vegetable intake analyses) or vegetable intake (in fruit intake analyses) and for country (in pooled analyses) or cohort (in analyses for Finland and Italy) (model 2). Tests for trend were performed by assigning the integers 0, 1 and 2 to the tertiles of the intake variables. Pearson correlations between fruit and vegetable intake ranged from -0.01 to 0.24; i.e., low enough to combine these variables in one model. Because some studies indicate that (saturated) fat may be positively associated with lung cancer risk[6], energy intake in model 2 was replaced by intakes of fat or saturated fat to evaluate their confounding effect on the relation between fruit and vegetable intake and lung cancer mortality. No such effect was observed (data not shown).

Information on prevalence of total cancer at baseline was present, but not for lung cancer specifically. Therefore, to examine the potential confounding effect of (subclinical) lung cancer cases at baseline, analyses were repeated after excluding the lung cancer deaths within the first two (n=11) and five years (n=28) of follow-up. Since the exclusion of these cases did not affect the associations for fruit and vegetables in any material way, results are presented for all cases. Also restricting the follow-up time to 20 years did not substantially change the results for fruit and for vegetable intake (data not shown).

To explore the shape of the relationship between fruit and vegetable intake and lung cancer mortality, rates expressed as number of lung cancer deaths per 10,000 person-years per tertile of intake were plotted. These rates were directly standardized for age and number of cigarettes smoked at baseline using the total population of current cigarette smokers as reference group.

To examine whether the associations with fruit and vegetable intake differed by smoking intensity, baseline smokers were categorized in light smokers, i.e., 1-19 cigarettes per day, and heavy smokers, i.e., 20 or more cigarettes per day. Moreover, never and former smokers were combined to nonsmokers. Limited cases in the latter two groups did not allow us to separate them in the analyses. Joint effects of smoking intensity (non, light and heavy smokers) and country-specific tertiles of intake were calculated but only for the countries pooled because of small number of cases per country.
All analyses were performed using the Statistical Analysis Systems (SAS) software package (version 6.12).

Results

Roughly half of the men in each country smoked cigarettes around 1970. Prevalences of never and former smokers differed per country. The mean number of cigarettes smoked was highest in Finland and lowest in Italy. Age at baseline was around 59 years on average, with baseline smokers being slightly younger and leaner (Table 1). Dietary intake varied across the countries, although within a country, smokers and nonsmokers did not differ substantially on intake, except for intake of fruit, with nonsmokers consuming more fruits (Table 1).
During 25 years of follow-up, 187 men died from lung cancer, of which 149 baseline smokers, 28 former smokers and 10 never smokers. The lung cancer mortality rate among smokers was highest in The Netherlands (84.2 per 10,000 person-years), followed by Finland (74.9 per 10,000 person-years) and lowest in Italy (35.6 per 10,000 person-years) (Table 1)

When pooling the populations, fruit consumption was inversely associated with lung cancer mortality in smokers (p trend = 0.05) (Table 2). Adjustment for potential confounders did not materially change the risk estimates, although statistical significance was no longer reached. Analyses for smokers per country showed a statistically significant inverse association for the Dutch cohort only (adjusted p trend = 0.004). In Finland, relative risks were below unity but not statistically significant, and in Italy no association was found. In contrast to fruit, the fresh fruit consumption in Finland showed only a nonsignificant lower risk in the highest tertile: RRs (adjusted model2, 95% CI) 1.00; 1.18 (0.66-2.11); 0.79 (0.41-1.51). Pooling the effect of fresh fruit across the three countries resulted in the following RRs (95% CI), adjusted according to model 2: 1.00; 0.83 (0.56-1.22); 0.70 (0.47-1.06); with a p trend of 0.09. Vegetable consumption was not related to lung cancer mortality in smokers (Table 2).

Table 1 Baseline characteristics and daily intake (mean ± SD) of male nonsmokers (never and former smokers) and of male smokers in the Finnish, Italian, and Dutch cohorts studied, and mortality during 25 years of follow-up

	Finland		Italy		The Netherlands	
	Nonsmokers (n = 651)	Smokers (n = 637)	Nonsmokers (n = 591)	Smokers (n = 616)	Nonsmokers (n = 288)	Smokers (n = 325)
Age (years)	59.4 ± 5.5	58.7 ± 5.5	59.8± 5.0	59.0 ± 4.9	60.4 ± 5.4	58.7 ± 5.2
Former smokers (%/total)	31.2		20.0		39.5	
Never smokers (%/total)	19.3		29.0		7.5	
Number of cigarettes daily		17.5 ± 9.0		11.7 ± 7.7		15.0 ± 8.5
Body mass index (kg/m^2)	25.8 ± 3.7	23.7 ± 3.7	27.1 ± 3.8	25.0 ± 3.8	25.4 ± 2.7	24.8 ± 2.8
Energy (MJ)	15.3 ± 4.6	15.7 ± 4.8	12.1 ± 3.5	12.3 ± 3.1	10.8 ± 2.2	11.0 ± 2.3
Fat (En%)	36.7 ± 6.7	38.0 ± 6.9	28.1 ± 8.0	28.0 ± 7.6	40.5 ± 5.6	41.4 ± 5.1
Saturated fat (En%)	21.3 ± 4.6	22.2 ± 4.7	9.3 ± 3.7	9.6 ± 3.6	16.5 ± 3.0	16.8 ± 2.8
Fruit (g)	186 ± 195	172 ± 173	162 ± 181	137 ± 146	192 ± 146	147 ± 110
Fresh fruit (g)	87 ± 84	72 ± 74	161 ± 181	137 ± 145	190 ± 145	144 ± 109
Vegetables (g)	80 ± 57	79 ± 63	71 ± 51	64 ± 55	184 ± 60	177 ± 57
Potatoes (g)	283 ± 142	288 ± 148	24 ± 23	22 ± 21	186 ± 102	191 ± 97
Total mortality (n)	457	532	378	446	195	233
Person-years	11,141	9,076	10,146	10,105	4,960	5,346
Total cancer mortality (n)	94	142	100	152	61	88
Lung cancer mortality (n)	17	68	5	36	16	45
former smokers	12		1		15	
never smokers	5		4		1	
Lung cancer mortality rate (per 10,000 person-years)		74.9		35.6		84.2

Table 2 Relative risks and 95% confidence intervals of 25-year lung cancer mortality according to fruit and vegetable consumption in country-specific tertiles in smoking men aged 50-69 years, pooled and per country

	Fruit Consumption				Vegetable Consumption			
	Low	Intermediate	High	*p* trend	Low	Intermediate	High	*p* trend
	Populations pooled (country-specific tertiles)				Populations pooled (country-specific tertiles)			
Cases (n)/total (n)	62/523	40/529	47/526		56/528	43/522	50/528	
Person-years	7,770	8,368	8,389		7,770	8,281	8,476	
Mortality rate (n/10,000 py)	79.8	47.8	56.0		72.1	51.9	59.0	
RR crude	1	0.59 (0.40-0.88)	0.69 (0.47-1.00)	0.05	1	0.71 (0.48-1.05)	0.80 (0.54-1.17)	0.24
RR adjusted model 1[1]	1	0.58 (0.39-0.86)	0.70 (0.48-1.02)	0.05	1	0.76 (0.51-1.13)	0.92 (0.63-1.36)	0.66
RR adjusted model 2[2]	1	0.56 (0.37-0.84)	0.69 (0.46-1.02)	0.05	1	0.73 (0.49-1.09)	0.90 (0.61-1.33)	0.59
	Finland				Finland			
Cases (n)/total (n)	27/212	19/213	22/212		26/213	18/212	24/212	
Person-years	2,857	3,113	3,105		2,946	3,037	3,092	
Mortality rate (n/10,000 py)	94.5	61.0	70.8		88.2	59.3	77.6	
Median intake (g/d) (range)	44.4 (0-79.8)	118.1 (80.2-177.6)	289.1 (177.8-1221)		25.7 (0-44.7)	62.4 (45.2-91.2)	131 (91.6-530.8)	
RR crude	1	0.63 (0.35-1.13)	0.74 (0.42-1.30)	0.29	1	0.66 (0.36-1.21)	0.87 (0.50-1.52)	0.63
RR adjusted model 1[1]	1	0.61 (0.34-1.09)	0.71 (0.40-1.25)	0.23	1	0.71 (0.39-1.30)	0.98 (0.56-1.72)	0.93
RR adjusted model 2[2]	1	0.63 (0.34-1.14)	0.82 (0.45-1.50)	0.50	1	0.69 (0.38-1.27)	0.99 (0.56-1.78)	0.96
	Italy				Italy			
Cases (n)/total (n)	12/203	10/207	14/206		14/205	10/202	12/209	
Person-years	3,294	3,412	3,400		3,040	3,341	3,724	
Mortality rate (n/10,000 py)	36.4	29.3	41.2		46.1	29.9	32.2	
Median intake (g/d) (range)	5 (0-48)	100 (50-158)	247.5 (159-1000)		18 (0-37)	55 (38-74)	98 (75-392)	
RR crude	1	0.81 (0.35-1.87)	1.14 (0.53-2.47)	0.72	1	0.64 (0.28-1.43)	0.65 (0.30-1.40)	0.28
RR adjusted model 1[1]	1	0.79 (0.34-1.84)	1.12 (0.52-2.44)	0.75	1	0.81 (0.36-1.84)	1.02 (0.46-2.26)	0.98
RR adjusted model 2[2]	1	0.79 (0.33-1.89)	1.08 (0.45-2.62)	0.85	1	0.83 (0.36-1.90)	1.05 (0.47-2.35)	0.92
	The Netherlands				The Netherlands			
Cases (n)/total (n)	23/108	11/109	11/108		16/110	15/108	14/107	
Person-years	1,619	1,844	1,883		1,784	1,903	1,659	
Mortality rate (n/10,000 py)	142.0	59.7	58.5		89.7	78.8	84.6	
Median intake (g/d) (range)	43 (0-93)	130 (94-175)	241.5 (179-850)		126.5 (0-154)	175 (155-194)	223 (195-530)	
RR crude	1	0.39 (0.19-0.80)	0.39 (0.19-0.79)	0.006	1	0.84 (0.42-1.71)	0.95 (0.46-1.95)	0.87
RR adjusted model 1[1]	1	0.40 (0.19-0.82)	0.42 (0.20-0.88)	0.01	1	0.82 (0.40-1.66)	0.92 (0.45-1.88)	0.80
RR adjusted model 2[2]	1	0.33 (0.16-0.70)	0.35 (0.16-0.74)	0.004	1	0.82 (0.40-1.66)	0.88 (0.43-1.82)	0.73

[1] adjusted for age, number of cigarettes smoked at baseline; [2] adjusted for age, number of cigarettes smoked at baseline, country (for pooled analysis)/cohort (for Finland/Italy), energy intake and vegetable intake (for fruit)/fruit intake (for vegetable)

We plotted country-specific lung cancer mortality rates in smokers against tertiles of fruit intake (median), standardized for age and numbers of cigarettes per day (Figure 1). This figure indicates that among smokers, fruit intake may be only inversely associated with lung cancer mortality in those with low intake and at high absolute risk. The absolute risk was low in Italy and did not change with fruit intake, as we observed using relative risks. For the Zutphen cohort and to a lesser extent for the Finnish cohorts, the absolute risks decreased with intake, in a way suggestive for a log-linear relationship.

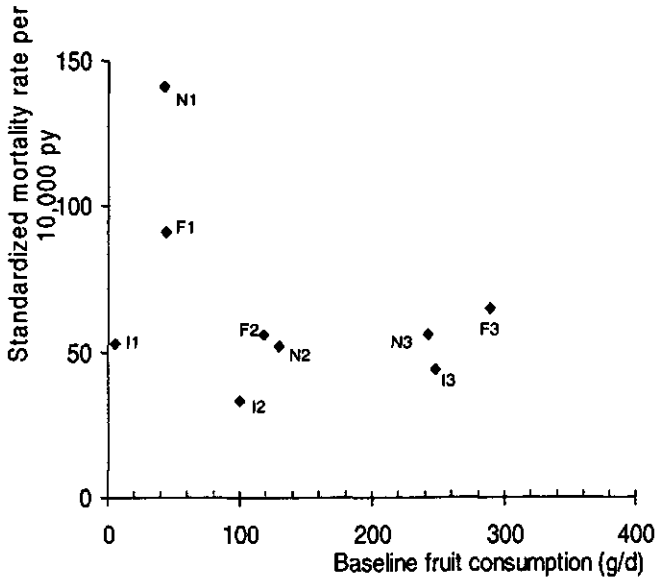
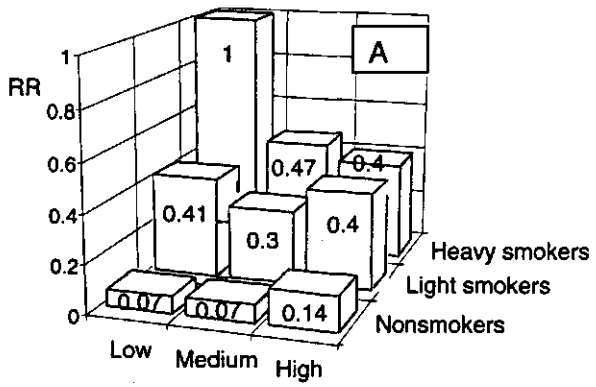


Figure 1 Lung cancer mortality rates standardized for age and numbers of cigarettes according to median of fruit intake in country-specific tertiles in smoking men aged 50-69 years followed for 25 years; F1, lowest tertile Finland; F2, intermediate tertile Finland; F3, highest tertile Finland; I1, lowest tertile Italy; I2, intermediate tertile Italy; I3, highest tertile Italy; N1, lowest tertile The Netherlands; N2, intermediate tertile The Netherlands; N3, highest tertile The Netherlands

In Figure 2A, the joint effects of cigarette smoking intensity (non, light and heavy) and fruit intake adjusted for age, energy intake, vegetable intake and country are shown. An inverse association was observed in the heavy smokers (RR (95% CI): 1; 0.47 (0.26-0.84); 0.40 (0.20-0.78)); in the light smokers no relation was seen. Figure 2B consists of the same figure for vegetable intake. An inverse association in the heavy smokers was indicated, however, risk estimates did not reach statistical

significance (RR (95% CI): 1; 0.92 (0.52-1.65); 0.64 (0.34-1.22)). The lung cancer risk among nonsmokers was about 10% of the risk in the reference group of heavy smokers with a low fruit/vegetable intake.



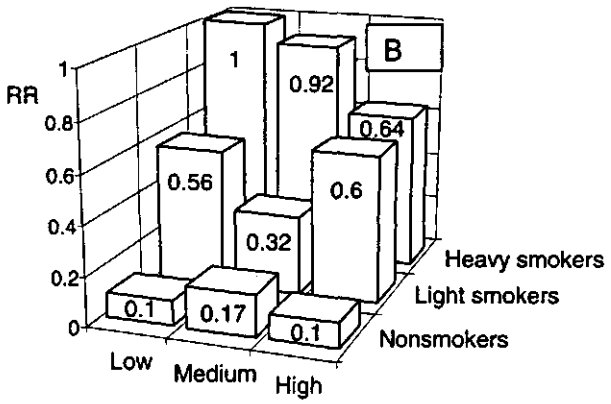


Figure 2 Relative risks of 25-year lung cancer mortality according to country-specific tertiles of fruit (A) and vegetable intake (B) and smoking status, adjusted for age, country, energy intake and vegetable and fruit intake, respectively

Discussion

In this prospective study among European smoking men, fruit intake was inversely related to lung cancer mortality. This association was particularly present in

the Dutch cohort, and in the heavy smokers. Vegetable intake was not related to lung cancer in our study. However, in heavy smokers, there was an indication for a decreased risk with higher intakes.

Although our results may not be fully comparable because we only examined smokers, they are in line with some but not all cohort studies. Fraser et al.[27] and Knekt et al.[28] observed an inverse association for fruit but not for vegetables, although the latter study did find an inverse relation for a vegetable subgroup, i.e., root vegetables. Moreover, the nonsignificant associations observed by Chow et al.[29] were suggestive for a protective effect of fruit but not of vegetables. In contrast, Steinmetz et al.[16] observed a statistically significant inverse relation for vegetable but not for fruit consumption, but she studied women only. Kvåle et al.[30] observed associations for both fruit and vegetables. Others only reported associations for fruit[31], for fruit and vegetables combined[15] or for specific fruits and vegetables[32]. Most case-control studies conducted[6-8] did find an inverse association between lung cancer and fruit and/or vegetable intake, among them also an Italian study[33].

As discussed in the introduction, results by smoking status are not consistent. Other cohort studies[15,16,18] did not report stronger associations in heavier smokers, as we did. But some case-control studies did observe (stronger) associations in heavy smokers[12,13]. Because heavy smokers have presumably the highest exposure to carcinogens and thus are at the highest risk, it may be plausible that the greatest risk reduction can be achieved in this group. On the other hand, it has been shown that in the Finnish cohorts the lighter smokers in 1959 quit smoking during follow-up more often than heavier smokers[34]. It is unclear to what extent this phenomenon played a role in the time period we studied and in the other cohorts included. However, we did check whether light smokers consumed more fruits and vegetables at baseline because this may have lead to bias. This was not the case (data not shown).

The cross-check dietary history we used is assumed to be valid for measuring the habitual intake[35]. The reproducibility was investigated for the Zutphen cohort and was satisfactory[36], with Spearman correlation coefficients of 0.65 for fruit and 0.50 for vegetables. However, assessment of fruit and even more of vegetable consumption is generally considered to be difficult[37].

A priori, fruit consumption was expected to be highest in the Italian cohorts. Total fruit consumption was, however, in the same range in the three countries, although intake of fresh fruits was lower in Finland. We think that the cohorts studied are not representative for the whole country, particularly due to the specific region and type of the cohorts. In the rural cohorts, availability of fruit mainly depended on the

production in the own region. While in Zutphen, fruits were mainly available by trade and thus less dependent of season. Because season of data collection differed, this may have also lead to less accurate measurements of the actual consumption level. However, we have no reasons to believe that the ranking of subjects, i.e., used in our analyses, is affected.

It is possible that we observed an association for fruit and not for vegetable consumption because of a larger variation in fruit intake. Another reason could be the fairly crude way, i.e., in food groups, in which we examined fruit and vegetable intake. The contents of these food groups and preparation methods could have differed between countries. We saw, for instance, that Knekt et al.[28] observed an association for a vegetable subgroup but not for all vegetables combined. On the other hand, Voorrips et al.[11] found no specific vegetables or fruits responsible for the association observed for total vegetable or fruit intake. The way our dietary information was computerized stopped us from constructing equal subgroups of fruits and vegetables for the three countries. Further, by using country-specific tertiles for analyzing the total population, we compared men with low intakes to those with high intakes in the specific countries, ignoring the exact level of consumption. We did this to prevent overrepresentation of countries in one of the tertiles. For the country-specific analyses, the number of lung cancer deaths were small, giving uncertain risk estimates with broad confidence intervals. This complicates interpreting differences in results between countries as real differences, for instance, between Finland and The Netherlands. The results from Italy, however, seem quite different. In this country, the absolute lung cancer mortality was much lower compared to the other two countries. Perhaps such findings indicate that only higher absolute risks may be lowered by fruit consumption.

We could use only one measurement to classify men as low, intermediate and high consumers. It is questionable how predictive a single measurement is. We know that during follow-up, changes in both diet and smoking habits occurred (for Finland see Pekkanen et al.[34]). Fruit and vegetable consumption increased in all three countries, mostly in Italy[20,38]. Prevention programs were carried out during this period; for instance, the North Karelia project in Finland[39]. Changes in exposure may have lead to misclassification, of which most is assumed to be random; generally such misclassification weakens associations. Moreover, the latency period of lung cancer is long, and restriction of follow-up time to 20 years did not change the results.

Residual confounding by smoking is of concern. By adjusting for intensity of smoking instead of just smoking status, we tried to limit this type of confounding. But we do not have all relevant information on smoking available, such as the age of

starting smoking and the type of tobacco smoked, therefore, residual confounding is still possible.

Although the relationship between fruit and vegetable consumption and lung cancer is generally assumed to be linear, our risk estimates of the highest intakes were somewhat closer to 1 than those of the intermediate intakes. This may be due to measurement error or chance, but it is also possible that the relation is not linear. One can expect that the protective effect levels off at a certain point. However, it is possible that before reaching this point the relationship is indeed linear. We tried to get some idea of the shape of the association between fruit consumption and lung cancer mortality by calculating absolute risks for tertiles of intake. This figure was suggestive for a log-linear association. It is questionable whether such statement can be based on the limited data points provided by our study. We believe that the shape of the relation deserves more attention in future research. Just comparing low and high intakes, levels that differ in each study, is an insufficient basis to refine the present general message to the public 'Eat more fruits and vegetables'.

Taken all evidence together[4-8,15,27] we cannot subscribe to the suggestion that results from cohort studies are less convincing than those from case-control studies[10]. Although not all risk estimates are statistically significant, most risk estimates, coming from various populations, are in the same, protective, direction. The difficulty in assessing fruit and vegetable intake, leading to misclassification, may be one reason for nonsignificant associations. Another reason may be the limited number of lung cancer cases in cohort studies, especially when subgroups are studied. Furthermore, since the number of cohort studies carried out is smaller compared to case-control studies, such comparison may be premature. However, we must take into consideration that future further disentanglement of the overwhelming effect of smoking from the effect of diet in lung cancer etiology may possibly result in weaker risk estimates for fruit and vegetable consumption.
In summary, fruit intake was inversely related to lung cancer mortality in this prospective study among European smoking men. This association was confined to the heavy smokers.

Acknowledgments

We are indebted to the many people involved in this longitudinal study, including all participants and the fieldwork teams in Finland, Italy and The Netherlands. We are grateful to the National Institute of Health in Rome for data collection since 1979. The study was supported by a grant of the Netherlands Prevention Foundation.

References

1. Parkin DM, Whelan SL, Ferlay J, et al. (Eds.). Cancer incidence in five continents, vol VII. IARC Scientific Publications No. 143. Lyon, France: IARC, 1997.
2. Berrino F, Sant M, Verdecchia A, Capocaccia R, Hakulinen T, Estève J (Eds.). Survival of cancer patients in Europe: the EUROCARE Study. : IARC Scientific Publications No. 132. Lyon, France: IARC, 1995.
3. Tomatis L, Aitio A, Day NE, Heseltine E, Kaldor J, Miller AB et al. (Eds.). Cancer: Causes, occurrence and control. IARC Scientific Publications No. 100. Lyon, France: 1990.
4. Steinmetz KA, Potter JD. Vegetables, fruit, and cancer. I. Epidemiology. Cancer Causes Control 1991; 2: 325-357.
5. Block G, Patterson B, Subar A. Fruit, vegetables, and cancer prevention: A review of the epidemiological evidence. Nutr Cancer 1992; 18: 1-29.
6. Ziegler RG, Mayne ST, Swanson CA. Nutrition and lung cancer. Cancer Causes Control 1996; 7: 157-177.
7. World Cancer Research Fund/American Institute for Cancer Research. Food, nutrition and the prevention of cancer: a global perspective. Washington, DC: American Institute for Cancer Research, 1997.
8. Committee on Medical Aspects of Food and Nutrition. Nutritional aspects of the development of cancer. Policy Report of the Working Group on Diet and Cancer of the Committee on Medical Aspects of Food and Nutrition Policy (COMA-report). Report on Health and Social Subjects, no. 48. London, UK: The Stationery Office, 1998.
9. Morabia A, Wynder EL. Dietary habits of smokers, people who never smoked, and exsmokers. Am J Clin Nutr 1990; 52: 933-937.
10. Koo LC. Diet and lung cancer 20+ years later: more questions than answers? Int J Cancer 1997; 73(Suppl 10): 22-29.
11. Voorrips LE, Goldbohm RA, Verhoeven DTH, Poppel van GAFC, Sturmans F, Hermus RJJ et al. Vegetable and fruit consumption and lung cancer risk in the Netherlands Cohort Study on Diet and Cancer. Cancer Causes Control 2000; 11: 101-115.
12. LeMarchand L, Yoshizawa CN, Kolonel LN, Hankin JH, Goodman MT. Vegetable consumption and lung cancer risk: a population-based case-control study in Hawaii. J Natl Cancer Inst 1989; 81: 1158-1164.
13. Ziegler RG, Mason TJ, Stemhagen A, Hoover R, Schoenberg JB, Gridley G. et al. Carotenoid intake, vegetables, and the risk of lung cancer among white men in New Jersey. Am J Epidemiol 1986; 123: 1080-1093.
14. Byers TE, Graham S, Haughey BP, Marshall JR, Swanson MK. Diet and lung cancer risk: findings from the Western New York diet study. Am J Epidemiol 1987; 125: 351-363.
15. Yong L-C, Brown CC, Schatzkin A, Dresser CM, Slesinski MJ, Cox CS et al. Intake of vitamins E, C, and A and risk of lung cancer. Am J Epidemiol 1997; 146: 231-243.
16. Steinmetz KA, Potter JD, Folsom AR. Vegetables, fruit, and lung cancer in the Iowa Women's Heath Study. Cancer Res 1993; 53: 536-543.
17. Mayne ST, Janerich DT, Greenwald P, Chorost S, Tucci C, Zaman MB et al. Dietary beta carotene and lung cancer risk in U.S. nonsmokers. J Natl Cancer Inst 1994; 86: 33-38.
18. Knekt P, Järvinen R, Seppänen R, Rissanen A, Aromaa A, Heinonen OP et al. Dietary

antioxidants and the risk of lung cancer. Am J Epidemiol 1991; 134: 471-479.
19. Keys A. (Ed.). Seven Countries: A multivariate analysis of death and coronary heart disease. Cambridge, MA: Harvard University Press, 1980: 1-381.
20. Alberti-Fidanza A, Fidanza F, Chiuchiù MP, Verducci G, Furttini D. Dietary studies on two rural Italian population groups of the Seven Countries Study. 3. Trend of food and nutrient intake from 1960 to 1991. Eur J Clin Nutr 1999; 53: 854-860.
21. Burke BS. The dietary history as a tool in research. J Am Diet Assoc 1947; 23: 1041-1046.
22. Pekkarinen M. Dietary surveys in connection with coronary heart disease studies in Finland. In: Bazan NC, Paoletti R, Lacono JM (Eds.). New trends in nutrition, lipid research, and cardiovascular diseases. Current topics in nutrition and disease, vol. 5. New York: A.R. Liss, 1981: 243-261.
23. Alberti-Fidanza A, Seccareccia F, Torsello S, Fidanza F. Diet of two rural population groups of middle-aged men in Italy. Int J Vitamin Nutr Res 1988; 58: 442-451.
24. Hautvast JGAJ. Commissie uniforme codering voedingsenquetes; ontwikkeling van een systeem om gegevens van voedingsenquetes met behulp van de computer te verwerken (In Dutch). Voeding 1975; 36: 356-361.
25. Keys A, Aravanis C, Blackburn HW, Buchem van FSP, Buzina R et al. Epidemiological studies related to coronary heart disease: characteristics of men aged 40-59 in seven countries. Acta Med Scand 1967; 460(Suppl): 1-392.
26. SAS Institute Inc. SAS technical report P-217, The PHREG procedure, version 6 Cary, NC, 1989.
27. Fraser GE, Beeson WL, Philips RL. Diet and lung cancer in California Seventh-day Adventists. Am J Epidemiol 1991; 133: 683-693.
28. Knekt P, Järvinen R, Teppo L, Aromaa A, Seppänen R. Role of various carotenoids in lung cancer prevention. J Natl Cancer Inst 1999; 91: 182-184.
29. Chow W-H, Schuman LM, McLaughlin JK, Bjelke E, Gridley G, Wacholder S et al. A cohort study of tobacco use, diet, occupation, and lung cancer mortality. Cancer Causes Control 1992; 3: 247-254.
30. Kvåle G, Bjelke E, Gart JJ. Dietary habits and lung cancer risk. Int J Cancer 1983; 31: 397-405.
31. Kromhout D. Essential micronutrients in relation to carcinogenesis. Am J Clin Nutr 1987; 45: 1361-1367.
32. Speizer FE, Colditz GA, Hunter DJ, Rosner B, Hennekens C. Prospective study of smoking, antioxidant intake, and lung cancer in middle-aged women (USA). Cancer Causes Control 1999; 10: 475-482.
33. Pisani P, Berrino F, Macaluso M, Pastorino U, Crosignani P, Baldasseroni A. Carrots, green vegetables and lung cancer: a case-control study. Int J Epidemiol 1986; 15: 463-468.
34. Pekkanen J, Tervahauta M, Nissinen A, Karvonen MJ. Does the predictive value of baseline coronary risk factors change over a 30-year follow-up? Cardiology 1993; 82: 181-190.
35. Block G. A review of validations of dietary assessment methods. Am J Epidemiol 1982; 115: 492-505.
36. Bloemberg BPM, Kromhout D, Obermann-de Boer GL, Kampen Van-Donker M. The

reproducibility of dietary intake data assessed with the cross-check dietary history method. Am J Epidemiol 1989; 130: 1047-1056.
37. Willett W, Lenart E. Reproducibility and validity of food-frequency questionnaires. In: W. Willett. Nutritional Epidemiology. Second edition. New York/Oxford: Oxford University Press, 1998: 101-147.
38. Huijbregts PPCW, Feskens EJM, Räsänen L, Alberti-Fidanza A, Mutanen M, Fidanza F et al. Dietary intake in five aging cohorts of men in Finland, Italy and the Netherlands. Eur J Clin Nutr 1995; 49: 852-860.
39. Puska P, Tuomilehto J, Nissinen A, Vartiainen E (Eds.). The North Karelia Project. Helsinki, Finland: Helsinki University Printing House, 1995.

Chapter 5

Vegetable and fruit consumption and lung cancer incidence among Dutch adults

Margje CJF Jansen, H Bas Bueno-de-Mesquita, A Jeanne M van Loon, Marga C Ocké, Frans J Kok, Daan Kromhout

Submitted

Abstract

Our objective was to study the association between fruit and vegetable consumption and subsequent lung cancer incidence among Dutch adults. Because of the increase in adenocarcinoma incidence among men in many countries, including The Netherlands, this association was investigated for adenocarinomas and Kreyberg I tumors separately. Dietary intake, other lifestyle factors, socio-demographic characteristics, and history of diseases were assessed for men and women aged 20-59 in the period 1987-1991. Cancer incidence was determined by linkage with cancer registries up to 1997. Complete baseline information was available for 33,796 persons of whom 140 developed lung cancer. Relative risks (RR) and 95% confidence intervals (95% CI) were estimated for fruit and vegetable consumption by Cox proportional hazard analyses adjusted for potential confounders. Vegetable consumption was inversely associated with lung cancer incidence; adjusted RRs (95% CI) for quartiles of frequencies of intake were 1; 0.90 (0.59-1.37); 0.63 (0.39-1.03); 0.56 (0.33-0.95), respectively; p trend 0.01. This relationship was very strong for incidence of adenocarcinomas (p trend 0.003), whereas incidence of Kreyberg I tumors was not statistically significantly associated with vegetable intake (p trend 0.24). The age-adjusted relative risk for fruit intake was 0.29 (0.17-0.50) in the highest versus the lowest quartile. After adjustment for other potential confounders fruit consumption was no longer related to lung cancer, mainly due to adjustment for smoking.

In conclusion, a higher vegetable intake was associated with a substantially lower lung cancer risk, especially regarding adenocarcinomas, in a cohort of relatively young Dutch adults. For fruit consumption no relationship with lung cancer was observed.

Introduction

Observational studies strongly suggest that increased fruit and vegetable consumption is associated with a reduced lung cancer risk[1-5]. Although the overall picture seems clear, inconsistencies have been reported regarding different associations for fruits and vegetables, stratified on smoking status, and stratified on histological type. Some cohort studies reported inverse associations for vegetables but not or much weaker for fruits[6-8], others vice versa[9-12]. Some investigators found more pronounced associations in non-smokers[11], others in ex-smokers[7], current smokers[13] or light smokers[14]. Regarding histological type, some studies observed associations that were stronger or confined to squamous cell carcinomas[6], others to large cell carcinomas[7], Kreyberg group I tumors (squamous cell, large cell, and small

cell carcinoma)[13] or to adenocarcinomas (Kreyberg group II tumors)[10]. Whether there are differences by histological type is especially of interest because among men an increase in adenocarcinomas has been observed in many countries, including The United States and The Netherlands[15,16].

Some cohort studies reported (stronger) associations for specific types or groups of vegetables, like carrots[6,8], tomatoes[6], green leafy vegetables[7] or Brassica vegetables[13]. However, no specific type or group of fruits and/or vegetables seems consistently responsible for 'an overall vegetable effect'. Moreover, because in most studies no adjustment is made for consumption of other fruits and vegetables, it is unclear whether results can be attributed to the specific type studied.

Besides residual confounding by smoking, the reported inconsistencies may be due to differences in study populations regarding sex, age range, smoking habits and consequently different distributions of the histological tumor types. The latter is important because it is assumed that the role of smoking is greater in Kreyberg group I than in Kreyberg group II tumors[17]. Moreover, it is hypothesized that the rise in adenocarcinomas may be due to the increased use of low-tar filter cigarettes and the subsequent differences in inhalation behavior[15]. So changes in smoking habits may lead to changes in proportions of histological types of lung cancer. Because declining survival rates for patients with adenocarcinomas in The Netherlands were indicated[18], the potential role of fruits and vegetables in the prevention of adenocarcinomas is of special interest.

We studied the association between vegetable and fruit consumption and lung cancer incidence among 33,796 Dutch men and women aged 20-59 at baseline followed for 8.4 years on average. Our study population is special in the sense that relatively young men and women were included, and that their smoking habits reflect present Dutch smoking patterns. We studied the impact of fruits and vegetables in the total study population, in baseline smokers, and for the Kreyberg group I and II tumors separately. Moreover, we analyzed specific vegetables and fruits consumed for their association with lung cancer incidence.

Materials and methods

Study population

From 1987 to 1991, more than 36,000 men and women participated in the Monitoring Project on Cardiovascular Disease Risk Factors. A detailed description of this project is given elsewhere[19]. In brief, each year a new random sample of men

and women aged 20-59 years was selected from the municipal registries of three Dutch cities, i.e., Amsterdam, Maastricht and Doetinchem, and invited to participate. The overall response rate was 50% for men and 57% for women.

For persons who participated more than once in the monitoring study (n=1,097), the first record was used in our longitudinal analysis. The initial data set consisted of 35,491 persons. We excluded subjects from analysis for several reasons: because they could not be linked to a unique number in the National Population Database (n=24), data on vital status were missing (n=343), they did not agree on release of medical records from the general practitioner and were therefore not offered for linkage to the cancer registry (n=597), prevalence of cancer at baseline (except non-melanoma skin cancer and cervix in situ) (n=542), inadequate completeness of the dietary data (n=84), and missing smoking data (n=105). This left 33,796 subjects for analysis.
The Monitoring Project on Cardiovascular Disease Risk Factors was approved by the Medical Ethical Committee of the University of Leiden, The Netherlands in 1987.

Data collection

Subjects received a self-administered questionnaire to be completed at home and were invited for a physical examination at the Municipal Health Center. At the health center, participants gave informed consent and field workers checked the questionnaire for completeness, and if necessary, helped the participants with further completion[19]. Socio-demographic characteristics, various lifestyle behaviors and presence of (a history of) several chronic diseases, among others cancer prevalence, were asked for.

The questionnaire included a short semi-quantitative food frequency questionnaire to assess usual dietary intake[20]. Consumption frequencies of nine commonly used vegetables in The Netherlands (cabbage, Brussels sprouts, chicory, spinach, string beans/snap beans, carrots, beetroot, tomatoes and mixed salad) were assessed. For fruits, consumption frequency was asked for apple, orange and mandarin. Intake of fruit juices was not measured. Consumption frequency was determined in six categories ranging from never to more than 4x/week. Although consumption was asked for the season in which the fruits and vegetables were sold, the ranking of subjects on total vegetable and fruit intake was not influenced when information on seasonal availability of the vegetables and fruits was taken into account (Spearman correlations: for vegetables $r = 0.99$ and for fruits $r = 0.97$).

Consumption of all vegetables and all fruits was combined to total vegetable consumption and to total fruit consumption, respectively. Consumption of mixed

salad was not asked from the start of the study, i.e., January 1987, but from August 1987. To calculate the total vegetable consumption for the study period till August 1987, we replaced the missing values on mixed salad intake with the sex, 10-year age group and education level specific mean of the study population. Energy intake was calculated using the computerized Dutch food composition table from 1993[21].

Information on smoking habits was gathered extensively by the self-administered questionnaire. Participants were asked whether they ever smoked cigarettes, cigars and/or pipe regularly, at what age they started smoking, the number of years smoked, and the mean number of cigarettes they (used to) smoke per day. Some people smoked cigars and/or pipe, and had never smoked cigarettes. Because there is evidence that besides cigarette smoking also cigar and pipe smoking raise the lung cancer risk[22,23], the overall smoking status (never, ex and baseline smoker) was used for analysis. Moreover, we calculated the variable pack-years of cigarette smoking assuming 20 cigarettes per pack.

Data of the cancer registries and of the self-administered questionnaire were used to determine baseline cancer prevalence. In the questionnaire it was asked whether a medical doctor ever diagnosed asthma, chronic bronchitis or other lung diseases. Based on this information, a dummy variable for (history of) lung diseases was made. Information on family history of lung diseases was not gathered. Supplement use was asked in the categories never, now and then, only during winter and through the whole year. Because it was not possible to calculate quantities based on these categories, two dummy variables for any use were constructed: "vitamin use": use of vitamin supplements A/D, B, C or multivitamin; and "other supplement use": use of garlic supplements and/or the category 'other supplement use'. The highest level of education reached was categorized into primary school, lower vocational/lower general, intermediate vocational/intermediate general, and higher vocational/university.

Cancer incidence and vital status

For the period 1987 to the end of 1997, follow-up for incident cancer has been established by computerized record linkage with the Netherlands Cancer Registry (NeCaRe), a national registry of all malignant tumors diagnosed from 1989 onwards in people living in the Netherlands, and with the three regional cancer registries covering the three cities from which the participants originated (IKA for Amsterdam, IKL for Maastricht and IKO for Doetinchem). Completeness, data consistency and the possibility of duplicate records are extensively checked by computer programs[24]. Because data from the NeCaRe are complete only for the period 1989 to the end of 1996, additional information from the regional cancer registries was used. In the

period 1987-1988, for IKL co-operation was obtained from all hospitals in the area, i.e., completeness was 100%. For IKA, the completeness was estimated 60% in 1987 and 95% in 1988. For IKO, this percentage was 75 for both years. For 1997, data from the regional registries were 100% complete. The method used for linkage is based upon the linkage procedure developed by Van den Brandt et al.[25]. This linkage procedure is a two-stage process in which the initial computerized linkage using a linkage key (date of birth, first four characters of the family name and sex) is followed by visual inspection with additional information to separate the computer matches into true and false positives.
After on average 8.4 years of follow-up, 140 incident lung cancer cases were identified. Data on vital status were obtained through the National Population Data.

Statistical analyses

We used Cox proportional hazard analysis to study the associations between vegetable and fruit consumption and lung cancer incidence. Frequencies of intake of total vegetables and total fruit were analyzed in quartiles. Moreover, we studied frequencies of intake of all specific types of vegetables and fruits for their association with lung cancer incidence. Relative risks (RR) and 95% confidence intervals (95% CI) were calculated, with the lowest quartile or category as the reference, and tests for trend were performed. Statistical significance was determined by two-sided tests with a critical value of 0.05. Statistical Analysis Systems (SAS) software package (version 6.12) was used for all analyses.

All prevalent cancers other than non-melanoma skin cancer and cervix carcinoma in situ were excluded from the analyses. Survival analyses were done 1) adjusted for age, 2) adjusted for age, sex, smoking status, pack-years of cigarette smoking and (history of) lung diseases (model 1), and 3) adjusted for model 1 plus total energy intake, fruit intake (in analyses on vegetable intake) or vegetable intake (in analyses on fruit intake), vitamin supplement use, and other supplement use (model 2). Analyses for specific types of vegetables and fruits were adjusted in the same way, except that adjustment was made for all vegetables and fruits consumed minus the one studied. Spearman correlations between consumption frequencies of specific vegetable types and all other vegetables consumed combined ranged from 0.14 for beetroot to 0.42 for tomatoes. These correlations for fruit were higher and ranged from 0.47 for mandarins to 0.53 for oranges.

To investigate whether associations were modified by sex and smoking status, interaction terms were included in the model. To examine the potential confounding effect of subclinical lung cancer cases, we repeated the analyses after excluding the cases within the first two years of follow-up (n=21). To investigate whether

associations with vegetable and fruit intake differed for the Kreyberg group I and Kreyberg group II tumors, analyses were stratified on histological type. These analyses were performed for tertiles instead of quartiles of intake because of limited numbers per Kreyberg group.

Results

After an average follow-up time of 8.4 years, 140 incident lung cancer cases occurred. During this period, 737 subjects (2.2%) had died and 275 (0.8%) had emigrated. In Table 1, characteristics of the lung cancer cases and the non-cases are given. Cases were more often men, (heavier) smokers, older, had a lower level of education, more often reported lung diseases, and had a lower fruit and vegetable consumption. Energy intake, vitamin supplement use, and use of other supplements were comparable between the cases and the non-cases.

Table 1 Baseline characteristics of the incident lung cancer cases and the non-lung cancer cases from the Monitoring Project on Cardiovascular Disease Risk Factors, The Netherlands, 1987-1991

	Lung cancer cases (n = 140)	Non-lung cancer cases (n=33,656)
Sex (%)		
men	64.3	46.8
women	35.7	53.2
Age (years) (mean ± SD)	51.9 ± 6.1	41.1 ± 10.9
Education (highest level reached) (%)[1]		
primary school	51.1	20.7
lower	35.3	41.5
intermediate	7.9	20.6
higher	5.8	17.2
Overall smoking status (%)		
baseline smokers	82.1	42.2
ex-smokers	15.7	24.6
never smokers	2.1	33.2
Pack-years of cig. smoking (mean ± SD)		
among baseline smokers	32.2 ± 20.6	16.4 ± 13.9
among ex-smokers	45.3 ± 37.1	13.1 ± 14.1
Prevalence/history of lung diseases (%)	22.9	11.2
Energy intake (MJ/d) (mean ± SD)	7.3 ± 2.2	7.4 ± 2.1
Vegetable cons. (freq./d) (mean ± SD)	0.99 ± 0.63	1.13 ± 0.59
Fruit consumption (freq./d) (mean ± SD)	0.77 ± 0.66	1.03 ± 0.66
Supplement use (%)		
vitamins (any)	35.7	38.4
other supplements (any)	20.7	15.6

[1] 1 missing among lung cancer cases; 81 missings among non-lung cancer cases

Several characteristics are shown per quartile of vegetable and of fruit intake (Table 2). A high vegetable consumption was reported more frequently by women, by older persons, by never smokers and ex-smokers, and by smokers with a lower number of pack-years of cigarette smoking. Similar relations were observed with fruit intake, however, the association with age was not so clear; and smoking behavior was much stronger associated with intake of fruits than of vegetables. The Spearman correlation coefficient between fruit and vegetable intake was 0.32.

Table 2 Baseline characteristics per quartile of vegetable consumption and of fruit consumption in frequencies/day; Monitoring Project on Cardiovascular Disease Risk Factors, The Netherlands, 1987-1991

	Quartile of intake			
	1 (low)	2	3	4 (high)
	Vegetable Consumption			
Number of subjects	8,475	8,447	8,384	8,490
Mean intake ± SD (freq./d)	0.49 ± 0.14	0.86 ± 0.10	1.23 ± 0.12	1.93 ± 0.47
Sex (%)				
men	57.1	49.1	43.0	38.2
women	42.9	50.9	57.0	61.8
Age (mean ± SD)	40.4 ± 11.4	41.1 ± 11.0	41.3 ± 10.8	41.7 ± 10.5
Smoking status (%)				
baseline smokers	48.3	42.5	41.2	37.6
ex-smokers	21.3	24.9	25.3	26.7
never smokers	30.4	32.6	33.4	35.7
Pack-years of cigarette smoking (mean ± SD)				
baseline smokers	17.8 ± 14.9	17.0 ± 14.0	15.6 ± 13.6	15.2 ± 13.4
ex-smokers	14.9 ± 16.0	13.0 ± 13.7	12.7 ± 13.9	12.3 ± 13.8
	Fruit Consumption			
Number of subjects	8,588	7,441	9,025	8,742
Mean intake ± SD (freq./d)	0.20 ± 0.14	0.73 ± 0.15	1.24 ± 0.15	1.89 ± 0.25
Sex (%)				
men	57.2	50.5	44.1	36.4
women	42.8	49.5	55.9	63.6
Age (mean ± SD)	41.4 ± 10.9	40.9 ± 11.2	40.9 ± 10.8	41.3 ± 10.9
Smoking status (%)				
baseline smokers	56.0	43.7	38.5	32.0
ex-smokers	20.0	24.9	25.8	27.5
never smokers	24.0	31.4	35.7	40.6
Pack-years of cigarette smoking (mean ± SD)				
baseline smokers	19.6 ± 15.8	16.2 ± 13.5	14.5 ± 12.5	14.0 ± 12.3
ex-smokers	16.1 ± 16.5	13.8 ± 15.3	12.4 ± 13.4	11.4 ± 12.3

The results of the analyses of intake of total vegetables and of total fruits in relation to lung cancer risk are presented in Table 3. Adjusted for age, frequency of

vegetable intake was strongly inversely associated with lung cancer incidence: the relative risk was 0.39 (0.24-0.65) for the highest intake compared to the lowest and a significant trend over the quartiles was observed. Further adjustment for potential confounders weakened the association somewhat, but a higher vegetable intake remained statistically significantly associated with a lower lung cancer risk (p trend 0.01). Lung cancer risk in the highest quartile of vegetable intake was 44% lower than in the lowest quartile of intake. Adjusted for age, relative risks were strongly reduced with higher fruit intake. The relative risk of the highest versus the lowest quartile was 0.29 (95% CI 0.17-0.50). After adjustment for potential confounders however, this relationship was no longer statistically significant, mainly due to the confounding effect of smoking and to a lesser extent of vegetable intake. Further adjustment for education did not materially change our findings for vegetable or for fruit intake.

Exclusion of the cases in the first two years of follow-up resulted in comparable risk estimates for vegetable intake. The relative risks for fruit consumption became closer to unity (data not shown).

Table 3 Relative risks and 95% confidence intervals of lung cancer incidence according to quartiles of daily frequencies of vegetable consumption and fruit consumption; Monitoring Project on Cardiovascular Disease Risk Factors, The Netherlands, 1987-1997

	Quartile of intake				
	1 (low)	2	3	4 (high)	p trend
	Vegetable Consumption				
Cases (n)/total (n)	51/8,475	40/8,447	28/8,384	21/8,490	
Person-years	70,367	71,004	70,713	70,715	
Mean intake	0.49	0.86	1.23	1.93	
(range)	(0-0.69)	(0.69-1.03)	(1.03-1.45)	(1.45-6.43)	
RR age-adjusted	1	0.73 (0.49-1.11)	0.48 (0.30-0.77)	0.39 (0.24-0.65)	0.0001
RR adj. model 1[1]	1	0.89 (0.59-1.35)	0.62 (0.38-1.00)	0.54 (0.33-0.91)	0.008
RR adj. model 2[2]	1	0.90 (0.59-1.37)	0.63 (0.39-1.03)	0.56 (0.33-0.95)	0.01
	Fruit Consumption				
Cases (n)/total (n)	60/8,588	30/7,441	32/9,025	18/8,742	
Person-years	71,157	61,921	75,607	74,115	
Mean intake	0.20	0.73	1.24	1.89	
(range)	(0-0.43)	(0.45-0.92)	(1-1.50)	(1.57-2.14)	
RR age-adjusted	1	0.60 (0.39-0.93)	0.54 (0.35-0.83)	0.29 (0.17-0.50)	0.0001
RR adj. model 1[1]	1	0.85 (0.55-1.32)	0.93 (0.60-1.43)	0.62 (0.36-1.06)	0.12
RR adj. model 2[2]	1	0.88 (0.57-1.38)	1.00 (0.63-1.56)	0.69 (0.39-1.20)	0.26

[1] adjusted for age, sex, smoking status, pack-years of cigarette smoking and presence of (history of) lung diseases; [2] adjusted for age, sex, smoking status, pack-years of cigarette smoking and presence of (history of) lung diseases, energy intake, fruit intake or vegetable intake, vitamin supplement use, and other supplement use

Table 4 Relative risks and 95% confidence intervals of lung cancer incidence according to tertiles of daily frequencies of vegetable consumption and fruit consumption for Kreyberg I and Kreyberg II tumors separately; Monitoring Project on Cardiovascular Disease Risk Factors, The Netherlands, 1987-1997

	Tertile of intake			
	1 (low)	2	3 (high)	*p* trend
	Vegetable Consumption			
	Kreyberg I tumors			
Cases (n)/total (n)	30/11,245	34/11,245	16/11,246	
Person-years	93,734	94,684	94,047	
Mean intake	0.55	1.04	1.79	
(range)	(0-0.80)	(0.80-1.29)	(1.29-6.43)	
RR age-adjusted	1	0.98 (0.60-1.62)	0.52 (0.28-0.95)	0.03
RR adj. model 1[1]	1	1.17 (0.70-1.93)	0.68 (0.37-1.25)	0.21
RR adj. model 2[2]	1	1.16 (0.70-1.93)	0.68 (0.36-1.29)	0.24
	Kreyberg II tumors			
Cases (n)/total (n)	34/11,229	11/11,242	7/11,237	
Person-years	93,582	94,739	93,999	
Mean intake	0.55	1.04	1.79	
(range)	(0-0.80)	(0.80-1.29)	(1.29-6.43)	
RR age-adjusted	1	0.30 (0.15-0.60)	0.20 (0.09-0.45)	0.0001
RR adj. model 1[1]	1	0.37 (0.19-0.73)	0.27 (0.12-0.61)	0.002
RR adj. model 2[2]	1	0.38 (0.19-0.76)	0.28 (0.12-0.64)	0.003
	Fruit Consumption			
	Kreyberg I tumors			
Cases (n)/total (n)	42/11,005	20/11,946	18/10,785	
Person-years	91,336	99,801	91,328	
Mean intake	0.28	1.03	1.80	
(range)	(0-0.64)	(0.71-1.29)	(1.29-2.14)	
RR age-adjusted	1	0.45 (0.27-0.77)	0.41 (0.24-0.72)	0.002
RR adj. model 1[1]	1	0.70 (0.41-1.21)	0.86 (0.48-1.52)	0.59
RR adj. model 2[2]	1	0.72 (0.42-1.25)	0.86 (0.47-1.56)	0.62
	Kreyberg II tumors			
Cases (n)/total (n)	27/10,990	14/11,940	11/10,778	
Person-years	91,259	99,761	91,299	
Mean intake	0.28	1.03	1.80	
(range)	(0-0.64)	(0.71-1.29)	(1.29-2.14)	
RR age-adjusted	1	0.48 (0.25-0.92)	0.38 (0.19-0.76)	0.006
RR adj. model 1[1]	1	0.72 (0.38-1.38)	0.73 (0.36-1.50)	0.39
RR adj. model 2[2]	1	0.85 (0.44-1.66)	0.98 (0.46-2.05)	0.95

[1] adjusted for age, sex, smoking status, pack-years of cigarette smoking and presence of (history of) lung diseases; [2] adjusted for age, sex, smoking status, pack-years of cigarette smoking and presence of (history of) lung diseases, energy intake, fruit intake or vegetable intake, vitamin supplement use, and other supplement use

No formal interaction was observed between vegetable or fruit intake and sex or smoking status. However, because of inconsistent results by smoking status in the

literature, additional analyses were restricted to baseline smokers. These results for vegetable intake and for fruit intake were not materially different (data not shown).

Of the 140 lung cancer cases, 57% were Kreyberg group I tumors (20% squamous cell, 19% small cell, and 19% large cell carcinoma), 37% Kreyberg group II tumors (adenocarcinoma), and 6% were other tumors. Results of analyses stratified by the Kreyberg group are given in Table 4. Incidence of Kreyberg group I tumors was not statistically significantly associated with vegetable intake, whereas the adenocarcinomas (Kreyberg II) were strongly inversely related with vegetable consumption: the model 2-adjusted relative risk (95% CI) in the highest tertile of intake was 0.28 (0.12-0.64). Fruit consumption was not associated with lung cancer incidence of Kreyberg group I nor of Kreyberg group II tumors.

Associations adjusted according to model 2 between intake of specific vegetables and lung cancer risk per histological tumor type are given in Table 5. For the Kreyberg group I tumors, chicory was inversely associated with risk with borderline significance. Higher intake of string beans/snap beans was related with a lower incidence of Kreyberg group II tumors. Moreover, higher intakes of cabbage and carrots were nonsignificantly associated with a lower Kreyberg group II incidence. Intakes of oranges, mandarins and apples were not associated with the Kreyberg group I tumors or the Kreyberg group II tumors after adjustment for potential confounders (data not shown).

When age-adjusted analyses were performed for all lung cancer cases, intakes of mixed salad, tomatoes, carrots, apples, mandarins, oranges, and with borderline significance cabbage and string beans/snap beans were inversely associated to risk. However, after adjustment for the other potential confounders and the other vegetables and fruits eaten, no specific vegetable or fruit was associated with overall lung cancer incidence (data not shown).

Discussion

This prospective study showed an inverse association between vegetable intake and lung cancer incidence. Especially the risk of adenocarcinomas was lowered by vegetable consumption. After adjustment, mainly due to smoking, a higher fruit intake was no longer statistically significant associated with a lower lung cancer risk.

When only adjusted for age, both fruit and vegetable consumption were strongly inversely related with lung cancer incidence in our study. Further adjustment for the

Table 5 Relative risks and 95% confidence intervals of lung cancer incidence according to vegetable types for Kreyberg I and II tumors; Monitoring Project on Cardiovascular Disease Risk Factors, The Netherlands, 1987-1997. Adjusted according to model 2: age, sex, smoking status, pack-years of cigarette smoking and presence of (history of) lung diseases, energy intake, fruit intake, vegetable intake minus specific type, vitamin supplement use, and other supplement use

	Kreyberg group I tumors				Kreyberg group II tumors			
	<1/month	1-3/month	≥ 1/week	*p* trend	<1/month	1-3/month	≥ 1/week	*p* trend
Cabbage	1	0.86 (0.48-1.53)	0.95 (0.54-1.67)	0.86	1	0.69 (0.36-1.31)	0.57 (0.28-1.16)	0.12
Brussels sprouts	1	0.64 (0.36-1.13)	1.00 (0.58-1.70)	0.99	1	1.03 (0.54-1.97)	1.14 (0.56-2.31)	0.68
Chicory	1	0.54 (0.30-1.00)	0.63 (0.37-1.08)	0.09	1	1.05 (0.48-2.28)	1.57 (0.74-3.31)	0.24
Spinach	1	0.78 (0.45-1.38)	1.08 (0.63-1.87)	0.77	1	1.08 (0.56-2.11)	1.32 (0.65-2.65)	0.44
String/snap beans	1	0.97 (0.43-2.22)	1.33 (0.62-2.88)	0.46	1	0.65 (0.31-1.36)	0.43 (0.20-0.92)	0.03
Carrot	1	1.03 (0.59-1.81)	1.29 (0.73-2.29)	0.38	1	0.59 (0.31-1.12)	0.58 (0.28-1.21)	0.15
Beetroot	1	1.53 (0.93-2.50)	1.05 (0.54-2.05)	0.88	1	0.43 (0.19-0.96)	1.00 (0.47-2.13)	0.99
Tomato	1	1.05 (0.54-2.02)	0.84 (0.47-1.48)	0.54	1	1.14 (0.53-2.42)	0.86 (0.43-1.72)	0.66
Mixed salad1[1]	1	0.47 (0.20-1.08)	0.77 (0.45-1.32)	0.34	1	1.00 (0.43-2.30)	0.72 (0.34-1.52)	0.39

[1] Missing values between January 1987 and August 1987 were not replaced by the sex, 10-year age group and education level specific mean of the study population. Subsequently, analyses for Kreyberg group I tumors were based on 73 cases on a total of 31,369 subjects and for Kreyberg group II tumors on 42 cases on a total of 31,338 subjects

other potential confounders weakened both associations, but especially the one with fruit. Fruit consumption was more closely correlated with smoking (intensity) than vegetable consumption (see Table 2), as observed by others[26,27]. And although the correlation between fruit and vegetable intake was not that high, further adjustment for vegetable intake, often not performed by other investigators, further weakened the relationship. It can be discussed whether prevalence of (history of) lung diseases is a potential confounder or an intermediate. Repeating the analyses leaving out this variable did not change our results (data not shown). Our results seemed to be robust, because analyses excluding the cases in the first two years of follow-up, and restricting to baseline smokers were not materially different from those performed in the whole study population.

When analyzing the age-adjusted relationship between intake of specific vegetables and fruits and lung cancer incidence, several types were inversely associated with risk. After further adjustment, including other fruits and vegetable eaten, no association remained. Associations with types of vegetables and fruits differed by histological type. After full adjustment there was an indication for an inverse association of chicory with Kreyberg group I, and of string beans/snap beans and to lesser extent of cabbage and carrots with Kreyberg group II tumors. However, because the number of cases per histological type was limited, these results should be interpreted with caution.

The dietary assessment method used was designed to rank subjects on their intake and not to assess the exact amounts eaten. Our results should therefore be interpreted as differences in lung cancer risk between high and low intake rather than for a given difference in intake. The number of vegetables and fruits asked for in the questionnaire was limited. In the data of the Dutch National Food Consumption Survey 1992[28], intake of our nine vegetables covered 67% of the intake of all vegetables, whereas intake of apples, oranges and mandarins added up to 55% of the intake of all fruits.

In general, habitual vegetable intake is difficult to assess. Observed correlation coefficients with a reference method range from 0.19 to 0.60[29,30]. Relative validity for our questionnaire fits in this range: compared with a cross-check dietary history a correlation coefficient of 0.34 was observed[20] and compared with the mean of twelve 24-h recalls the Spearman correlation was 0.23 (unpublished results). Similar to other food frequency questionnaires (correlations in the range 0.34-0.72[29,30]), relative validity of fruit intake was better, with correlation coefficients of 0.66 against the cross-check dietary history and 0.39 against the 24-h recalls. Moreover, fruit and

vegetable intake was correlated with sex, age and smoking habits in the directions commonly found[26,27,31,32].
Because our food frequency questionnaire did not assess the consumption of all main dietary carbohydrate sources, levels of calculated energy intake were low. The relative validity in ranking subjects (against the 12 24-h recalls) was however good, i.e., $r = 0.71$ (unpublished results).

Because smoking is such a strong lung cancer determinant and is associated with decreased intake of fruits and vegetables, a residual confounding effect may be likely. In this study, we collected detailed information on smoking and by adjusting both for smoking status and for pack-years of cigarette smoking, we tried to limit this effect. However, some residual confounding by smoking may still be present.

Cancer incidence was obtained through record linkage. The linkage key was found to have a sensitivity of 98% and an initial predictive value of a computer match of 98% in the Netherlands Cohort Study[25]. We may have missed some cases because the registration was not complete in 1987 and 1988, incidence in some years was based on the regional cancer registries only, and subjects were lost to follow-up due to emigration or were not linked due to several reasons. For persons not linked because of missings on a linkage to National Population Database, vital status or because they did not agree on release of medical records, mean age, smoking status and fruit and vegetable intake was calculated. These persons were somewhat younger (i.e. 38.6 years) than the non-lung cancer cases, but were to the same extent baseline smokers. Vegetable intake was slightly lower, probably because of the younger age.

Our study population consisted of relatively young men and women. We had more adenocarcinomas (37%) and less squamous cell tumors (20%) compared to the general Dutch population (22% and 43%, respectively), and consequently a lower prevalence of Kreyberg group I tumors (57% versus 70%)[33]. This observation is also in accordance with the observed increase in incidence of adenocarcinomas in The Netherlands[15]. Adenocarcinomas occur more often in women than in men[17] and were found to be more frequent in young male patients than in older men[34]. In our study population, however, the mean age and distribution of the sexes (data not shown) were comparable between both Kreyberg tumor groups. Smoking was slightly stronger associated with incidence of Kreyberg group I than of group II tumors. The relative risk (95% CI) for smoking status (in 3 categories) was 3.75 (2.24-6.28) and for pack-years of cigarette smoking 1.03 (1.02-1.04) for group I tumors in a model including age and sex. For Kreyberg group II tumors these figures were 3.41 (1.90-6.12) and 1.02 (1.01-1.04), respectively.

As mentioned in the Introduction, results of epidemiological studies by histological type are not consistent. In studies in which vegetable and fruit consumption was stronger associated with squamous and/or small cell carcinoma than with adenocarcinoma[6,13(for men),35], all or the majority of subjects were males and prevalence of smoking was relatively high. As in the present study, Steinmetz et al.[7] and Speizer et al.[8] found stronger relations for vegetables. These studies have in common that they investigated women only and that the prevalence of adenocarcinomas was relatively high (33 and 41%, respectively). Therefore, it seems that vegetable consumption may be more related to reduction of the lung cancer risk in study populations that include women, that have a relatively high prevalence of adenocarcinomas, and that have a relatively lower exposure to cigarette smoke. However, findings from Fraser et al.[10] do not fit this picture, and thus show that inconsistencies still remain.

In conclusion, in this prospective study among relatively young Dutch adults, vegetable intake was inversely related with lung cancer incidence. This association was mainly observed with adenocarcinomas. After adjustment for confounders, fruit intake was no longer related to lung cancer risk.

Acknowledgments

The Monitoring Project on Cardiovascular Disease Risk Factors was financially supported by the Ministry of Public Health, Welfare and Sports of The Netherlands. GL Obermann-de Boer is kindly acknowledged for coordinating the study and epidemiologists and field-workers of the Municipal Health Services in Amsterdam, Doetinchem and Maastricht for their important contribution to the data collection. We are thankful to LJ Schouten, O Visser and J van Dijk of the cancer registries Netherlands Cancer Registry/IKL, IKA and IKO, respectively for their support in the linkage with the cancer registries. A Blokstra and PE Steinberger from the National Institute of Public Health and the Environment are thanked for data management. We thank LE Frank for her excellent help in preparing the cancer data for analysis.

References

1. Steinmetz KA, Potter JD. Vegetables, fruit, and cancer. I. Epidemiology. Cancer Causes Control 1991; 2: 325-357.
2. Block G, Patterson B, Subar A. Fruit, vegetables, and cancer prevention: A review of the epidemiological evidence. Nutr Cancer 1992; 18: 1-29.
3. Ziegler RG, Mayne ST, Swanson CA. Nutrition and lung cancer. Cancer Causes Control 1996; 7: 157-177.
4. World Cancer Research Fund/American Institute for Cancer Research. Food, nutrition

and the prevention of cancer: a global perspective. Washington, DC: American Institute for Cancer Research, 1997: 143-147.
5. Report of the Working Group on Diet and Cancer of the Committee on Medical Aspects of Food and Nutrition Policy (COMA-report). Nutritional aspects of the development of cancer. Report on Health and Social Subjects, no. 48. London, UK: The Stationery Office, 1998.
6. Kvåle G, Bjelke E, Gart JJ. Dietary habits and lung cancer risk. Int J Cancer 1983; 31: 397-405.
7. Steinmetz KA, Potter JD, Folsom AR. Vegetables, fruit, and lung cancer in the Iowa Women's Health Study. Cancer Res 1993; 53: 536-543.
8. Speizer FE, Colditz GA, Hunter DJ, Rosner B, Hennekens C. Prospective study of smoking, antioxidant intake, and lung cancer in middle-aged women (USA). Cancer Causes Control 1999; 10: 475-482.
9. Kromhout D. Essential micronutrients in relation to carcinogenesis. Am J Clin Nutr 1987; 45: 1361-1367.
10. Fraser GE, Beeson WL, Philips RL. Diet and lung cancer in California Seventh-day Adventists. Am J Epidemiol 1991; 133: 683-693.
11. Knekt P, Järvinen R, Seppänen R, Rissanen A, Aromaa A, Heinonen OP, Albanes D, Heinonen M, Pukkala E, Teppo L. Dietary antioxidants and the risk of lung cancer. Am J Epidemiol 1991; 134: 471-479.
12. Chow W-H, Schuman LM, McLaughlin JK, Bjelke E, Gridley G, Wacholder S, Chien HTC, Blot WJ. A cohort study of tobacco use, diet, occupation, and lung cancer mortality. Cancer Causes Control 1992; 3: 247-254.
13. Voorrips LE, Goldbohm RA, Verhoeven DTH, Poppel van GAFC, Sturmans F, Hermus RJJ, Brandt van den PA. Vegetable and fruit consumption and lung cancer risk in the Netherlands Cohort Study on Diet and Cancer. Cancer Causes Control 2000; 11: 101-115.
14. Yong L-C, Brown CC, Schatzkin A, Dresser CM, Slesinski MJ, Cox CS, Taylor PR. Intake of vitamins E, C, and A and risk of lung cancer. The NHANES I Epidemiologic Followup Study. Am J Epidemiol 1997; 146: 231-243.
15. Janssen-Heijnen MLG, Coebergh JWW, Klinkhamer PJJM, Schipper RM, Splinter TAW, Mooi WJ. Is there a common etiology for the rising incidence of and decreasing survival with adenocarcinoma of the lung? Epidemiology 2001; 12: 256-258.
16. Devesa SS, Shaw CL, Blot WJ. Changing patterns of lung cancer incidence by histological type. Cancer Epidemiol Biomarkers Prev 1991; 1: 29-34.
17. Schottenfeld D, Fraumeni JF (Eds.). Cancer epidemiology and prevention. Second edition. New York/Oxford: Oxford university press, 1996: 640-642.
18. Janssen-Heijnen MLG, Schipper RM, Klinkhamer PJJM, Crommelin MA, Mooi WJ, Coebergh JWW. Divergent changes in survival for histological types of non-small-cell lung cancer in southeastern Netherlands since 1975. Br J Cancer 1998; 77: 2053-2057.
19. Verschuren WMM, Leer van EM, Blokstra A, Seidell JC, Smit HA, Bueno de Mesquita HB, Oberman-de Boer GL, Kromhout D. Cardiovascular disease risk factors in the Netherlands. Neth J Cardiol 1993; 6: 205-210.
20. Bloemberg BPM, Kromhout D, Jansen AM, Goddijn HE. Reproducibility and validity of a short self-administered semi-quantitative food frequency questionnaire. In: Bloemberg

BPM. On the effect of measurement error in nutritional epidemiology using dietary history and food frequency methodology (Thesis). Leiden, The Netherlands: Leiden University, 1993: 45-65.
21. Stichting NEVO. Nederlands voedingsstoffenbestand 1993 (In Dutch). The Hague: Voorlichtingsbureau voor de Voeding, brochure 202, 1993.
22. Boffetta P, Pershagen G, Jöckel K-H, Forastiere F, Gaborieau V, Heinrich J et al. Cigar and pipe smoking and lung cancer risk: a multicenter study from Europe. J Natl Cancer Inst 1999; 91: 697-701.
23. Shapiro JA, Jacobs EJ, Thun MJ. Cigar smoking in men and risk of death from tobacco-related cancers. J Natl Cancer Inst 2000; 92: 333-337.
24. Winter de GA, Coebergh JWW, Leeuwen van FE, Schouten LJ (Eds.). Incidence of cancer in The Netherlands 1989. First report of the Netherlands Cancer Registry. Utrecht, The Netherlands: Hoonte-Holland bv, 1990.
25. Brandt van den PA, Schouten LJ, Goldbohm RA, Dorant E, Hunen PMH. Development of a record linkage protocol for use in the Dutch cancer registry for epidemiological research. Int J Epidemiol 1990; 19: 553-558.
26. Zondervan KT, Ocké MC, Smit HE, Seidell JC. Do dietary and supplementary intakes of antioxidants differ with smoking status? Int J Epidemiol 1996; 25: 70-79.
27. Morabia A, Wynder EL. Dietary habits of smokers, people who never smoked, and exsmokers. Am J Clin Nutr 1990; 52: 933-937.
28. Anonymous. Zo eet Nederland, 1992. Resultaten van de voedselconsumptiepeiling 1992 (In Dutch). Den Haag: Voorlichtingsbureau voor de Voeding, 1993.
29. Ocké MC, Bueno-de-Mesquita HB, Goddijn HE, Jansen AM, Pols MA, Staveren van WA, Kromhout D. The Dutch EPIC food frequency questionnaire. I. Description of the questionnaire, and relative validity and reproducibility for food groups. Int J Epidemiol 1997; 26(Suppl 1): S37-S48.
30. Kaaks R, Slimani N, Riboli E. Pilot phase studies on the accuracy of dietary intake measurements in the EPIC Project: Overall evaluation of results. Int J Epidemiol 1997; 26(Suppl 1): S26-S36.
31. Patterson BH, Block G, Rosenberger WF, Pee D, Kahle LL. Fruit and vegetables in the American diet: Data from the NHANES II Survey. Am J Public Health 1990; 80: 1443-1449.
32. Thompson RL, Margetts BM, Speller VM, McVey D. The Health Education Authority's health and lifestyle survey 1993: who are the low fruit and vegetable consumers? J Epidemiol Community Health 1999; 53: 294-299.
33. Bergman L, Zandwijk van N, Schaake-Koning CCE, Coebergh JWW, Leeuwen van FE. Longkanker. In: Ruwaard D, Kramers PGN (Eds.). Volksgezondheid Toekomst Verkenning (In Dutch). Den Haag, The Netherlands: Sdu Uitgeverij Platijnstraat, 1993: 273-279.
34. Kreuzer M, Kreienbrock L, Gerken M, Heinrich J, Bruske-Hohlfeld I, Muller K-M, Wichmann HE. Risk factors for lung cancer in young adults. Am J Epidemiol 1998; 147: 1028-1037.
35. Byers TE, Graham S, Haughey BP, Marshall JR, Swanson MK. Diet and lung cancer risk: findings from the Western New York Diet Study. Am J Epidemiol 1987; 125: 351-363.

Chapter 6

Quantity and variety of fruit and vegetable consumption and cancer risk

Margje CJF Jansen, H Bas Bueno-de-Mesquita, Edith JM Feskens, Frans J Kok, Daan Kromhout

Submitted

Abstract

The recommendation for fruit and vegetable intake includes a certain *quantity* and eating a *variety*. The evidence for eating a *variety* is limited. We examined the association between cancer risk and adherence to the recommendation for fruits and vegetables, addressing both the aspect of *quantity* and *variety*, in a prospective cohort study among 730 Dutch men aged 65-84 years. These men were followed for 10 years resulting in 138 cancer cases. The *quantity* of fruits and vegetables was assessed using a dietary history, while the *variety* in intake was based on a food frequency questionnaire.

Adherence to the recommended amounts of fruit and vegetables was inversely associated with total cancer risk: the relative risk (RR) adjusted for potential confounders was 0.56 (95% confidence interval (CI) 0.31-1.00). Eating the recommended daily 200 grams of vegetables was not related to cancer incidence, whereas eating the recommended 200 grams of fruit was associated with a 38% lower risk compared to eating less than 100 grams. Variety in vegetable intake was inversely associated with total cancer and with non-lung epithelial cancer: the RRs (95% CI) for the highest vs. lowest tertile were 0.64 (0.43-0.95) and 0.51 (0.27-0.97), respectively. Variety in fruit intake was not associated with cancer risk.

In conclusion, adherence to the guidelines for fruit and vegetable intake was associated with lower cancer risk. Besides *quantity*, also *variety* in intake is of importance.

Introduction

Dietary recommendations have been originally formulated to meet nutrient requirements and to prevent deficiency diseases. Nowadays prevention from chronic diseases like cancer is used as an additional rationale to promote a healthy diet. Fruits and vegetables are an important source of many required nutrients, and epidemiological studies have consistently shown that fruit and vegetable consumption is inversely associated with especially the risk of epithelial cancers[1,2]. It is unclear whether specific fruits, vegetables or compounds are responsible for this relation.

In 1991 the "5 A Day for Better Health" Program was started in the US to promote an increase in fruit and vegetable consumption[3]. Other countries followed, among others The Netherlands in 1995. The Dutch recommendation for fruit and vegetable intake aims at a certain *quantity*, i.e., 200 grams of fruits and 150-200 grams of vegetables daily, and at *variety* in consumption. The association between the

amount or frequency of fruit and vegetable intake and cancer risk has been extensively studied, mostly in middle-aged persons[1,2]. For variety, on the contrary, the evidence is limited[4,5]. In most case-control studies, variety in vegetable intake was inversely associated with cancer risk[6-11], but not in all[12,13]; whereas variety in fruit intake was inversely related to cancer risk in only two of these studies[9,10]. In the only prospective study conducted, variety in vegetable intake was not independently associated with lung cancer risk, while variety in fruit intake was not related with lower risk of lung cancer[14].

We examined whether fruit and vegetable intake according to the guidelines, examining both the aspect of *quantity* and *variety*, was associated with total cancer risk in a cohort of elderly men. Moreover, we specified analyses to lung cancer and non-lung epithelial cancer. Since the aspect of variety has been hardly studied in prospective studies, we focused on this aspect of the dietary guidelines.

Subjects and methods

Study population

The study population consisted of men participating in the Zutphen Elderly Study, an extension of the Zutphen Study. In 1960, the Zutphen Study started with a cohort of 878 men from Zutphen (The Netherlands) born between 1900 and 1919, as the Dutch contribution to the Seven Countries Study[15]. In 1985, 367 of the 555 participants who were still alive were re-examined. In addition, a random sample (two out of three) of all other men in the same age range living in Zutphen was invited to participate in the study (n=711). In total, 939 men (response rate 74%) were examined in 1985, and complete information on dietary and other risk factors was available for 790 men. After excluding all prevalent cancer cases, 730 men remained for analysis.

Data collection

Dietary and medical examinations were carried out between March and June 1985. The habitual diet in the month preceding the interview was determined using a cross-check dietary history method adapted to the Dutch situation[16]. Experienced dietitians interviewed participants at home in the presence of the person usually preparing meals. A checklist of foods and quantities of food bought per week was used to calculate and verify the participant's food consumption pattern. The dietitians encoded the food intake data, and these data were converted into energy and nutrient data using the 1985 release of the Dutch food composition table. We used the dietary history-data to compute the amount of fruits and vegetables

consumed in daily grams. Further, a frequency questionnaire assessed the habitual intake of 27 vegetable types and 7 fruit types. The vegetables assessed were those commonly eaten in The Netherlands (a list is available on request), whereas the fruits measured were mainly available and eaten in summer, i.e., strawberries, berries, grapes, peaches, cherries, prunes and apricots. Consumption was asked in five categories ranging from never to more than once a week in the season that these foods were on the market. We calculated the variety in fruit and vegetable consumption by summing up the number of vegetables and fruits eaten at least once a month based on the food frequency questionnaire only. We did not include potatoes in the vegetable group.

Detailed information on smoking behavior was obtained by a questionnaire, by which smoking status (never, ex, baseline) and pack-years of cigarette smoking could be determined. Weight and height were measured while participants wore underwear only, and body mass index was calculated (weight/height^2). The total minutes of physical activity per week (engaged in walking, cycling, hobbies, sports, gardening and work) derived from a self-administered questionnaire originally designed for retired men[17] were calculated. Socio-economic status (SES) was determined by job status, based on the job performed longest[18] and categorized in four groups, i.e., professionals/managers/teachers (highest), small business owners, non-manual workers, and manual workers (lowest).

Follow-up and disease ascertainment

Information on the vital status of the participants until January 1995 was obtained from municipal population registries. Three men were lost to follow-up in 1991. These men had moved, of which two abroad and one with unknown destination. They were included in the analyses, but censored at 31 December 1990.

Disease prevalence at baseline and incidence of disease during follow-up was recorded during the examinations in 1985, 1990, 1993, and 1995 using standardized questionnaires. All information was verified with hospital discharge data and written information from the general practitioner. Three physicians coded the incidence uniformly using the 9th Revision of the International Classification of Diseases[19].
We studied incident total cancer (ICD 140-209), incident lung cancer (ICD 162) and incident epithelial cancer other than lung (i.e., tumors of the oropharynx, esophagus, stomach, colon, rectum, liver, gallbladder, pancreas, kidney and bladder) as endpoints. Since it is often difficult to determine the underlying cause of death in elderly people, we included both the primary and secondary cause of death in the analyses. At baseline, 60 men (8%) had cancer and we excluded them from the analyses.

Statistical analysis

We used Cox proportional hazard analysis (SAS procedure PHREG, SAS release 6.12, SAS Institute, North Carolina, USA) to study associations between vegetable and fruit consumption, both *quantity* and *variety*, and cancer incidence. To study *quantity*, we divided the fruit and vegetable consumption into three groups based on the Dutch dietary guidelines: **group 1**: <150 g/d of vegetables (i.e., less than the lower limit) and/or <100 g/d of fruits (less than half the recommendation); **group 3**: ≥200 g/d of vegetables and ≥200 g/d of fruits (meeting both the recommendation for vegetables (upper limit) and for fruit); and **group 2**: those remaining. We also studied the vegetable and fruit intakes separately by degree of adherence to the guidelines. We divided vegetable intake into <150, 150-200 and 200+ g/d, and fruit intake in <100, 100-200 and 200+ g/d. We studied *variety* in consumption in tertiles of intake. We adjusted the analyses for age only, and for age, smoking status, pack-years of cigarette smoking, total energy intake, alcohol intake, physical activity, and body mass index (BMI). Moreover, when we analyzed fruit intake, we made an additional adjustment for vegetable intake and similarly we adjusted vegetable intake for fruit intake. When we studied variety, we made an additional adjustment for amount of fruit and vegetables consumed. We could make these additional adjustments because the Spearman correlation coefficient between fruit and vegetable intake was 0.13, and between quantity and variety 0.23, 0.25, 0.30, for fruit plus vegetables, vegetables and fruit, respectively. In addition, we adjusted all analyses for SES. We derived probability values for a linear trend from tertile medians.

Results

During 10 years of follow-up 138 men developed cancer. Of these, 97 men had epithelial cancer, including cancers of the oropharynx (n=7), esophagus (n=2), stomach (n=7), colon (n=16), rectum (n=3), liver/gall bladder/pancreas (n=9), kidney/bladder (n=16), and lung/bronchus (n=42). Numbers do not add up to 97 because 5 men had more than one incident tumor. Only the first occurring cancer has been included in the analyses.

Because the focus of this paper is on *variety* in intake, we give descriptors of the study population by tertiles of variety in fruit and vegetable intake (Table 1). Men in the lowest tertile of variety tended to have a lower socio-economic status, to be more often smokers, slightly older, to consume less fruit and vegetables, to have a lower energy intake, and to be less physical active compared to men in the highest tertile of variety.

Table 1 Baseline characteristics of the study population by tertile of variety in fruit and vegetable consumption, the Zutphen Elderly Study 1985

	Tertiles of variety (range)		
	Low (3-18)	Medium (19-23)	High (24-33)
Number of men	232	258	240
SES (%)[1]			
professionals	18.3	24.9	34.6
small-business owners	21.0	21.8	16.7
non-manual workers	28.4	27.2	26.7
manual workers	32.3	26.1	22.1
Smoking status (%)			
never	22.0	18.2	18.3
ex	43.5	50.8	54.2
current	34.5	31.0	27.5
	Mean (SD)		
Variety (# types)	14.8 ± 3.2	21.1 ± 1.4	26.6 ± 2.4
Age (years)	71.5 ± 5.5	71.0 ± 5.2	70.8 ± 4.9
Vegetable intake (g/d)	158 ± 77	179 ± 66	193 ± 73
Fruit intake (g/d)	184 ± 150	194 ± 134	224 ± 136
Energy intake (MJ/d)	9.2 ± 2.3	9.6 ± 2.1	9.7 ± 2.1
Alcohol intake (g/d)	11.6 ± 16.3	13.7 ± 17.3	14.3 ± 17.4
Pack-years of cigarette smoking	24.2 ± 22.7	20.3 ± 23.1	20.4 ± 15.6
among ex-smokers	29.2 ± 16.4	29.5 ± 14.6	33.3 ± 20.7
among baseline smokers			
Total physical activity (min/wk)	590 ± 590	712 ± 651	773 ± 700
BMI (kg/m^2)	25.7 ± 3.1	25.6 ± 3.2	25.5 ± 3.1

[1] n=3 missings in lowest tertile and n=1 missing in medium tertile

Sixteen percent of the study population met both the recommendations for the quantity of vegetable and of fruit intake. The lowest category of quantity of fruit and vegetable consumption consisted of 48% of the study population, and the middle category of 36% of the study population (Table 2). Consuming fruits and vegetables according to the guidelines was associated with a 44% lower total cancer risk (Table 2). Variety in fruit and vegetable intake was not related to cancer risk, although relative risks decreased with higher variety (Table 2).

In Table 3, the risk estimates for the association between vegetable intake and incidence of total cancer, lung cancer, and non-lung epithelial cancer are shown. The quantity of vegetables consumed was not related to cancer incidence, whereas the variety in vegetable intake was inversely associated with total cancer risk. The highest tertile of variety in vegetable intake was associated with a 36% lower total cancer risk. Additional adjustment for grams of fruits and vegetables consumed did not weaken the association. The association observed for total cancer was mainly due to the relation found for incidence of non-lung epithelial cancers.

Table 2 Age-adjusted and multiple-adjusted relative risks (RR) for total cancer incidence by adherence to the Dutch guidelines for fruit and vegetable intake (*group 1*: <150 g/d of vegetables and/or <100 g/d of fruit; *group 2*: not in group 1 or group 3; *group 3*: ≥200 g/d of vegetables and ≥200 g/d of fruit) and by tertiles of variety in fruit and vegetable intake, the Zutphen Elderly Study 1985-1995

	Vegetable and fruit intake by adherence to the guidelines			
	Group 1	Group 2	Group 3	*p* trend
Cases (*n*)/Total (*n*)	76/350	48/266	14/114	
Intake (g/d) (mean ± SD)	282 ± 127	407 ± 102	601 ± 165	
Age-adjusted RR (95% CI)	1	0.78 (0.54-1.12)	0.53 (0.30-0.94)	0.02
Adjusted RR (95% CI)[1]	1	0.79 (0.55-1.14)	0.56 (0.31-1.00)	0.03
	Tertiles of variety in vegetable and fruit intake			
	Low	Medium	High	*p* trend
Cases (n)/Total (n)	49/232	52/258	37/240	
Variety (mean ± SD)	14.8 ± 3.2	21.1 ± 1.4	26.6 ± 2.4	
Age-adjusted RR (95% CI)	1	0.94 (0.63-1.38)	0.74 (0.48-1.13)	0.16
Adjusted RR (95% CI)[1]	1	0.90 (0.61-1.34)	0.71 (0.46-1.09)	0.12
Adjusted RR (95% CI)[2]	1	0.93 (0.63-1.39)	0.75 (0.48-1.16)	0.19

[1] adjusted for age, smoking status, pack-years of cigarette smoking, total energy intake, physical activity, BMI and alcohol intake; [2] adjusted for age, smoking status, pack-years of cigarette smoking, total energy intake, physical activity, BMI, alcohol intake, and fruit and vegetable intake

Associations between fruit intake, quantity and variety, and cancer risk are given in Table 4. Meeting the recommended fruit intake, i.e., 200 g/d, was associated with a 38% lower cancer incidence compared to those eating less than 100 g/d. Both incidences of lung cancer and non-lung epithelial cancer were lower with higher fruit intake, but associations did not reach statistical significance. The risk estimates for higher variety in fruit intake were all below 1, but none was statistically significant. Additional adjustment for fruit and vegetable intake slightly weakened the association between total cancer and variety in fruit intake (Table 4).

Further adjustment for SES did not materially change the results (data not shown). Moreover, excluding the cancer cases in the first two years of follow-up (n=29) did not lead to different results for the quantity of vegetables or fruits, nor for the variety in vegetable consumption (data not shown). For variety in fruit intake, however, associations with total cancer and non-lung epithelial cancer became statistically significant: RRs (95% CI), 0.63 (0.41-0.96), 0.65 (0.39-1.09), *p* trend 0.03; and 0.51 (0.26-1.00), 0.54 (0.24-1.20), *p* trend 0.04, respectively. Also the association between variety in fruit and vegetable intake and total cancer became slightly stronger, i.e., of borderline significance: RRs (95% CI), 0.89 (0.57-1.39), 0.64 (0.39-1.05), *p* trend 0.08.

Table 3 Age-adjusted and multiple covariate-adjusted relative risks (RR) for incidence of total cancer, lung cancer and non-lung epithelial cancer by degree of adherence to the Dutch guidelines for vegetable intake and to tertiles of variety in vegetable intake, the Zutphen Elderly Study 1985-1995

	Vegetable intake (g/d)				Tertiles of variety in vegetable intake			
	0-150	150-200	200+	*p* trend	Low	Medium	High	*p* trend
Total cancer								
Cases (n)/Total (n)	57/270	43/240	38/220		65/273	31/198	42/259	
Mean intake; variety	112 ± 29	173 ± 14	260 ± 66		12.3 ± 3.0	17.1 ± 0.8	21.2 ± 2.0	
Age-adjusted RR (95% CI)	1	0.85 (0.57-1.26)	0.78 (0.51-1.17)	0.22	1	0.64 (0.42-0.99)	0.66 (0.45-0.97)	0.03
Adjusted RR (95% CI)[1]	1	0.84 (0.56-1.25)	0.80 (0.53-1.20)	0.28	1	0.62 (0.41-0.96)	0.63 (0.43-0.93)	0.02
Adjusted RR (95% CI)[2]	1	0.87 (0.58-1.29)	0.83 (0.54-1.25)	0.36	1	0.61 (0.40-0.95)	0.64 (0.43-0.95)	0.02
Lung cancer								
Cases (n)/Total (n)	16/270	15/240	11/220		20/273	7/198	15/259	
Age-adjusted RR (95% CI)	1	1.07 (0.53-2.16)	0.82 (0.38-1.76)	0.61	1	0.48 (0.20-1.12)	0.79 (0.40-1.54)	0.44
Adjusted RR (95% CI)[1]	1	1.14 (0.56-2.32)	0.90 (0.41-1.94)	0.79	1	0.47 (0.20-1.11)	0.77 (0.39-1.52)	0.42
Adjusted RR (95% CI)[2]	1	1.22 (0.59-2.49)	0.95 (0.44-2.07)	0.92	1	0.47 (0.20-1.13)	0.82 (0.41-1.63)	0.53
Non-lung epithelial cancer								
Cases (n)/Total (n)	25/270	17/240	15/220		27/273	15/198	15/259	
Age-adjusted RR (95% CI)	1	0.75 (0.40-1.39)	0.70 (0.37-1.33)	0.27	1	0.74 (0.39-1.39)	0.56 (0.30-1.05)	0.07
Adjusted RR (95% CI)[1]	1	0.73 (0.39-1.37)	0.73 (0.38-1.39)	0.33	1	0.72 (0.38-1.36)	0.54 (0.28-1.01)	0.05
Adjusted RR (95% CI)[2]	1	0.73 (0.39-1.37)	0.73 (0.38-1.39)	0.33	1	0.69 (0.36-1.30)	0.51 (0.27-0.97)	0.04

[1] adjusted for age at baseline, smoking status, pack-years of cigarette smoking, total energy intake, physical activity, BMI and alcohol intake; [2] adjusted for age at baseline, smoking status, pack-years of cigarette smoking, total energy intake, physical activity, BMI, alcohol intake, fruit intake, and vegetable intake when variety studied

Table 4 Age-adjusted and multiple covariate-adjusted relative risks (RR) for incidence of total cancer, lung cancer and non-lung epithelial cancer by degree of adherence to the Dutch guidelines for fruit intake and to tertiles of variety in fruit intake, the Zutphen Elderly Study 1985-1995

	Fruit intake (g/d)				Tertiles of variety in fruit intake			
	0-100	100-200	200+	*p* trend	Low	Medium	High	*p* trend
Total cancer								
Cases (n)/Total (n)	38/153	49/251	51/326		57/241	49/303	32/186	
Mean (± SD) intake; variety	41 ± 29	144 ± 27	319 ± 123		1.9 ± 1.1	4.6 ± 0.5	6.3 ± 0.4	
Age-adjusted RR (95% CI)	1	0.72 (0.47-1.10)	0.57 (0.37-0.87)	0.01	1	0.69 (0.47-1.01)	0.75 (0.48-1.15)	0.07
Adjusted RR (95% CI)[1]	1	0.77 (0.50-1.18)	0.62 (0.40-0.95)	0.03	1	0.70 (0.48-1.02)	0.75 (0.49-1.17)	0.07
Adjusted RR (95% CI)[2]	1	0.77 (0.50-1.18)	0.62 (0.40-0.96)	0.04	1	0.72 (0.49-1.07)	0.81 (0.52-1.28)	0.15
Lung cancer								
Cases (n)/Total (n)	13/153	16/251	13/326		17/241	16/303	9/186	
Age-adjusted RR (95% CI)	1	0.71 (0.34-1.48)	0.44 (0.20-0.94)	0.03	1	0.76 (0.39-1.51)	0.70 (0.31-1.58)	0.33
Adjusted RR (95% CI)[1]	1	0.84 (0.39-1.77)	0.56 (0.25-1.26)	0.15	1	0.79 (0.40-1.58)	0.75 (0.33-1.71)	0.43
Adjusted RR (95% CI)[2]	1	0.84 (0.40-1.79)	0.58 (0.26-1.29)	0.17	1	0.86 (0.43-1.71)	0.88 (0.38-2.05)	0.67
Non-lung epithelial cancer								
Cases (n)/Total (n)	18/153	16/251	23/326		25/241	19/303	13/186	
Age-adjusted RR (95% CI)	1	0.52 (0.27-1.03)	0.58 (0.31-1.07)	0.16	1	0.62 (0.34-1.12)	0.68 (0.35-1.34)	0.12
Adjusted RR (95% CI)[1]	1	0.56 (0.28-1.12)	0.64 (0.34-1.22)	0.29	1	0.63 (0.34-1.14)	0.72 (0.37-1.42)	0.16
Adjusted RR (95% CI)[2]	1	0.56 (0.28-1.11)	0.62 (0.33-1.19)	0.26	1	0.62 (0.34-1.13)	0.71 (0.35-1.42)	0.15

[1] adjusted for age at baseline, smoking status, pack-years of cigarette smoking, total energy intake, physical activity, BMI and alcohol intake; [2] Adjusted for age at baseline, smoking status, pack-years of cigarette smoking, total energy intake, physical activity, BMI, alcohol intake, fruit intake, and vegetable intake when variety studied

Discussion

In this population of elderly men, adherence to the recommended amounts of fruits and vegetables was associated with a 44% lower cancer risk during 10-year of follow-up. For fruit intake, the quantity was inversely related to cancer risk and not the variety. For vegetable intake, not the quantity but the variety was inversely associated with cancer incidence. The latter relation was mainly seen for the group of epithelial cancers other than lung cancer.

High intakes of fruits and vegetables have been associated with lower cancer risk at different sites in many, but not all, epidemiological studies. Some studies found stronger associations for fruits, others for vegetables[1,2]. We found an inverse association between total cancer and the consumed amount of fruits, but not of vegetables. Fruit intake was inversely related with both lung cancer and non-lung epithelial cancer, although no statistical significance was reached.
Variety is part of the dietary guidelines for a long time[4,5,20]. Besides its relation with nutritional adequacy[21,22], evidence for this recommendation is limited[4,5]. However, it is hypothesized that rather a mix of potentially plant-based anticarcinogens than a single compound may protect against cancer. Several indices of overall diet diversity or quality and diversity within food groups have been developed[23]. Such indices have been used to examine nutrient adequacy and dietary quality[23,24], and have been related to risk of chronic diseases[25-29].

In our study, variety in vegetable intake was inversely associated with total cancer and with non-lung epithelial cancer. Similar results were found for colorectal cancer[6-8], gastric cancer[9] and oropharyngeal cancer[10] in case-control studies. These studies were conducted in Italy and the latter in Switzerland. In two case-control studies on colon cancer from the US[12,13], a higher vegetable variety was nonsignificantly related with lower risk. Prospectively, variety in vegetable intake was not associated with lung cancer risk[14]. This is consistent with our results, because we did not observe an association for lung cancer.
Variety in fruit intake was not related to total cancer risk in our study. The reductions in lung cancer risk with higher fruit variety we (12%) and Feskanich et al.[14] (19%) observed were both not statistically significant. In case-control studies, variety in fruit intake was inversely related to gastric cancer[9] and to oropharyngeal cancer[10], but not to colon/colorectal cancer[6-8,12,13].

Most epidemiological studies use food frequency questionnaires (FFQ) to assess consumption. FFQs rank fruit and to a lesser extent vegetable intakes mostly reasonably well, however, they are less suitable to measure the level of intake

accurately[30]. Therefore, most studies compare a low with a high intake by dividing the consumption in tertiles, quartiles or quintiles, depending on the study size. These studies are less appropriate to study adherence to guidelines in terms of absolute levels and cancer risk. Our validated dietary history-method[16] assessed however grams of consumption, and was therefore suitable for this purpose. To ascertain variety in fruit and vegetable intake we used a food frequency questionnaire. Because correlations between variety and quantity did not exceed 0.3 in our study, probably because they originated from different sources, we could assess their associations independently. This is in contrast with the study of Feskanich et al.[14] in which quantity and variety were highly correlated. It may be questioned whether that study is suitable to assess independent associations for quantity (i.e. frequency) and variety.

In our study, vegetable variety was based on a wide range of commonly eaten vegetables in The Netherlands. For fruit variety, the number of items was smaller, and consisted of fruits consumed mainly during summer. We found variety in vegetable intake more strongly related to cancer risk than the quantity consumed, and for fruit intake vice versa. However, we must point at the smaller range in quantity and at the wider range in variety of vegetable consumption compared to fruit intake. Also in the case-control studies performed on this subject, the range of variety in fruit intake was mostly smaller than of vegetable intake[6-13]. Therefore, it is difficult to conclude that fruit variety is not associated with cancer risk. Especially, because after excluding the cases in the first two years of follow-up, this association became statistically significant. This may be an indication that also variety in fruit intake plays a role in reducing the cancer risk.

An advantage of our study is its prospective design. However, subjects could have changed their habits due to subclinical disease at baseline. We checked this influence by excluding the cases in the first two years of follow-up. The associations for quantity of intake did not differ, and the relations with variety in intake became stronger. The latter was also observed by McCullough et al.[27] with the Healthy Eating Index. Moreover, subjects could have changed their lifestyle during follow-up. Because cancer is a disease with a long latency period, we do not expect that such changes have had a major impact on outcome. A disadvantage of our study was the small sample size, especially for performing subgroup analyses. Moreover, at baseline the men had a mean age of 71. So, dietary intake and other lifestyle factors before baseline may have influenced the cancer risk.

We saw that the men in the highest tertile of variety in fruit and vegetable intake also had the healthiest lifestyle (regarding smoking, physical activity and dietary

aspects) and the lowest risk profile (regarding age and SES). This is consistent with a study on total diet diversity based on the NHANES I-data[25]. Education and energy intake were higher with higher diversity, while male smokers and those little physically active had a low diversity. Also Slattery et al.[13] found younger and higher educated individuals having a more diverse diet. The question is whether variety in intake itself is the reason for the lower risk or just a marker for those at the lowest risk. By adjusting for potential confounders we tried to estimate the association for variety itself, however, we cannot rule out residual confounding.

To summarize, adherence to the dietary guidelines regarding fruit and vegetable intake was associated with a substantially lower cancer risk in this elderly male population. Regarding fruit intake, quantity was associated with a reduction in cancer incidence. For vegetable intake, higher variety and not quantity lead to lower cancer incidence, mainly of non-lung epithelial cancers. Moreover, because we do not know which constituents of fruits and vegetables are responsible for reducing the cancer risk, it seems wise to stress the aspect of variety in the dietary recommendations to the public.

References

1. Steinmetz KA, Potter JD. Vegetables, fruit, and cancer. I. Epidemiology. Cancer Causes Control 1991; 2: 325-357.
2. World Cancer Research Fund/American Institute for Cancer Research. Food, nutrition and the prevention of cancer: a global perspective. Washington, DC: American Institute for Cancer Research, 1997.
3. Heimendinger J, Van Duyn MA, Chapelsky D, Foerster S, Stables G. The national 5 A Day for Better Health Program: a large-scale nutrition intervention. J Public Health Manag Pract 1996; 2: 27-35.
4. Haughton B, Gussow JD, Dodds JM. An historical study of the underlying assumptions for United States food guides from 1917 through the basic four food group guide. J Nutr Educ 1987; 19: 169-176.
5. National Research Council. Recommended dietary allowances. Tenth edition. Washington, DC: National Academy Press, 1989.
6. Fernandez E, D'Avanzo B, Negri E, Franceschi S, La Vecchia C. Diet diversity and the risk of colorectal cancer in Northern Italy. Cancer Epidemiol Biomarkers Prev 1996; 5: 433-436.
7. Franceschi S, Favero A, La Vecchia C et al. Food groups and risk of colorectal cancer in Italy. Int J Cancer 1997; 72: 56-61.
8. Fernandez E, Negri E, La Vecchia C, Franceschi S. Diet diversity and colorectal cancer. Prev Med 2000; 31: 11-14.
9. La Vecchia C, Munoz SE, Braga C, Fernandez E, Decarli A. Diet diversity and gastric cancer. Int J Cancer 1997; 72: 255-257.

10. Levi F, Pasche C, La Vecchia C, Lucchini F, Franceschi S, Monnier P. Food groups and risk of oral and pharyngeal cancer. Int J Cancer 1998; 77: 705-709.
11. Franceschi S, Favero A, La Vecchia C et al. Influence of food groups and food diversity on breast cancer risk in Italy. Int J Cancer 1995; 63: 785-789.
12. McCann SE, Randall E, Marshall JR, Graham S, Zielezny M, Freudenheim JL. Diet diversity and risk of colon cancer in Western New York. Nutr Cancer 1994; 21: 133-141.
13. Slattery ML, Berry D, Potter J, Caan B. Diet diversity, diet composition, and risk of colon cancer. Cancer Causes Control 1997; 8: 872-882.
14. Feskanich D, Ziegler RG, Michaud DS et al. Prospective study of fruit and vegetable consumption and risk of lung cancer among men and women. J Natl Cancer Inst 2000; 92: 1812-1823.
15. Keys A, Aravanis C, Blackburn H. Epidemiological studies related to coronary heart disease: characteristics of men aged 40-59 in seven countries. Acta Med Scand 1966; 460(Suppl): 1-392.
16. Bloemberg BPM, Kromhout D, Obermann-de Boer GL, van Kampen-Donker M. The reproducibility of dietary intake data assessed with the cross-check dietary history method. Am J Epidemiol 1989; 130: 1047-1056.
17. Caspersen CJ, Bloemberg BPM, Saris WHM, Merritt RK, Kromhout D. The prevalence of selected physical activities and their relation with coronary heart disease risk factors in elderly men: the Zutphen Study, 1985. Am J Epidemiol 1991; 133: 1078-1092.
18. Duijkers TJ, Kromhout D, Spruit IP, Doornbos G. Inter-mediating risk factors in the relation between socioeconomic status and 25-year mortality (the Zutphen Study). Int J Epidemiol 1989; 18: 658-662.
19. World Health Organization. Manual of the international statistical classification of diseases, injuries, and causes of death. Geneva: WHO, 1977.
20. Wolf JD, Peterkin BB. Dietary guidelines: the USDA perspective. Food Technology 1984; 38: 80-86.
21. Kant AK, Schatzkin A, Block G, Ziegler RG, Nestle M. Food group intake patterns and associated nutrient profiles of the US population. J Am Diet Assoc 1991; 91: 532-537.
22. Krebs-Smith SM, Smicklas-Wright H, Guthrie HA, Krebs-Smith J. The effect of variety in food choices and dietary quality. J Am Diet Assoc 1987; 87: 897-903.
23. Kant AK. Indexes of overall diet quality: A review. J Am Diet Assoc 1996; 96: 785-791.
24. Drewnowski A, Henderson SA, Shore AB, Fischler C, Preziosi P, Hercberg S. Diet quality and dietary diversity in France: Implications for the French paradox. J Am Diet Assoc 1996; 96: 663-669.
25. Kant AK, Schatzkin A, Harris TB, Ziegler RG, Block G. Dietary diversity and subsequent mortality in the First National Health and Nutrition Examination Survey Epidemiologic Follow-up Study. Am J Clin Nutr 1993; 57: 434-440.
26. Kant AK, Schatzkin A, Graubard BI, Schairer C. A prospective study of diet quality and mortality in women. JAMA 2000; 283: 2109-2115.
27. McCullough ML, Feskanich D, Stampfer MJ et al. Adherence to the dietary guidelines for Americans and risk of major chronic disease in women. Am J Clin Nutr 2000; 72: 1214-1222.
28. McCullough ML, Feskanich D, Rimm EB et al. Adherence to the dietary guidelines for Americans and risk of major chronic disease in men. Am J Clin Nutr 2000; 72: 1223-

1231.

29. Huijbregts P, Feskens E, Rasanen L et al. Dietary pattern and 20 year mortality in elderly men in Finland, Italy, and The Netherlands: longitudinal cohort study. BMJ 1997; 315: 13-17.
30. Krebs-Smith SM, Heimendinger J, Subar AF, Patterson BH, Pivonka E. Using food frequency questionnaires to estimate fruit and vegetables intake: association between the number of questions and total intakes. J Nutr Ed 1995; 27: 80-85.

Chapter 7

Plasma carotenoid levels in Dutch men and women, and the relation with vegetable and fruit consumption

Margje CJF Jansen, Anne Linda van Kappel, Marga C Ocké, Pieter van 't Veer, Elio Riboli, H Bas Bueno-de-Mesquita

Submitted

Abstract

Fruit and vegetable intake is inversely associated with cancer risk in many epidemiological studies. Accurate assessment of consumption of these foods is however difficult, and biomarkers of intake would overcome several drawbacks of currently used dietary assessment methods. Therefore, we investigated the relation between plasma carotenoids and usual vegetable and fruit intake. Plasma carotenoid concentrations were measured and vegetable, fruit and juice consumption was assessed by a food frequency questionnaire (FFQ) in a random sample of 591 Dutch men and women aged 20-59 years from the MORGEN-project, one of the contributions to the EPIC-study. In this sample of the general Dutch population, plasma β-cryptoxanthin was the best indicator for fruit intake, and for the sum of vegetable, fruit and juice intake. For a contrast between the medians of the lowest vs. highest quartile of this intake, the difference in plasma β-cryptoxanthin level was 49% and 55% in men, and 41% and 42% in women, respectively. In addition, plasma β-cryptoxanthin distinguished between three of the quartiles of this intake. Plasma lutein concentrations divided low from high vegetable consumers: levels differed 15% in men and 18% in women between the lowest and highest quartile of vegetable intake. Lutein concentrations could not separate all quartiles of vegetable intake.

In conclusion, plasma carotenoids were only crude indicators of vegetable and fruit intake as assessed by a FFQ; β-cryptoxanthin for fruit intake and lutein for vegetable intake. None of the plasma carotenoids could distinguish all four quartiles of vegetables, fruit and/or juice intake.

Introduction

Many epidemiological studies around the world have associated a higher fruit and vegetable intake with a lower cancer risk, mainly of epithelial cancers[1]. Till now it is not clear whether the total of vegetables, fruits and juices consumed, the intake of vegetables or of fruits, of specific vegetables or fruits, or of compounds in these products are responsible for this association.

The difficulty of assessing vegetable and fruit intake[2-4] complicates this kind of research. Methods used depend on the participant's memory and ability to take into account the variability in intake, for example from day to day, or by season. Moreover, memory might be biased by the actual health status. Measuring compound concentrations in human tissue indicative for intake, i.e., biomarkers of intake, would be an objective method to assess dietary intake. Carotenoids are candidates for biomarkers of vegetable and fruit intake, because these foods are the primary source

and carotenoids cannot be synthesized by humans or animals. Moreover, because carotenoids differ in type and in quantity between (varieties of) vegetables and fruits, it should be possible also to distinguish intake of vegetables and fruits separately. Carotenoids in blood or adipose tissue have been studied as biomarkers of intake of vegetables and fruits[5-7]. It seems that absolute intakes cannot be translated into carotenoid concentrations. Intakes and carotenoid concentrations are however correlated, although the observed correlation is generally not strong[3-5,8]. Besides dietary intake, also factors as sex, age, BMI, blood cholesterol, smoking and alcohol drinking have been associated with carotenoid concentrations[9,10].

Within the framework of the European Prospective Investigation into Cancer and Nutrition (EPIC)[11], van Kappel et al.[12] analyzed the plasma concentrations of six carotenoids in the 16 geographical areas of the EPIC-study in persons aged 45-64 years. In the present paper we studied one of these areas, i.e., The Netherlands, and used an extended sample of 591 Dutch men and women 20-59 years.
The EPIC-participants are followed over time to study the relations between dietary intake, among others of vegetables and fruits, and cancer risk. Both the total intake of vegetables, fruits and juices and the intake of vegetables and fruits separately or specific foods may be etiologically of importance. Therefore, we studied the associations between these intakes as assessed with a food frequency questionnaire and plasma carotenoid concentrations to investigate whether plasma levels could indicate intake. Because in epidemiological studies dietary intake is often categorized, we studied whether carotenoid concentrations were able to discriminate between quartiles of intake.

Methods

Study population

Our study population is a sub-sample of the participants of the MORGEN-project, which is one of the two Dutch contributions to the EPIC-study[11]. The MORGEN-project contains 22,769 respondents from a random sample of the Dutch population aged 20-64 years from three towns in the Netherlands (Amsterdam, Doetinchem, and Maastricht) examined in the period 1993 to 1997[13]. The response rate was 45%[14]. The Medical Ethical Committee of TNO Nutrition and Food Research approved the study according to the guidelines of the Helsinki Declaration.
Our study was an extension of a study conducted within the framework of EPIC[12]. In each of the 16 geographical areas of EPIC in 9 European countries, 100 men and 100 women were randomly selected among the participants aged 45-64 years with

complete data, with equal numbers in the sex-age strata. One of the areas was The Netherlands.
For the present study, the sample from the Dutch MORGEN-project has been extended to 36 participants per sex and 5y-age-stratum in the age range 20-59 with complete data. Within these strata, sampling was random, except that participants were equally selected from each town, i.e., Amsterdam, Doetinchem and Maastricht. From the 576 participants selected, one person had not given informed consent for the EPIC-study, for one person carotenoid analyses failed, and three persons had missing data on potential confounders, leaving 571 persons for data analysis.

Data collection

Participants of the MORGEN-project filled in two self-administered questionnaires: a general questionnaire and a semi-quantitative food frequency questionnaire. In addition, during a physical examination at the Municipal Health Service weight and height were measured and blood was collected by trained research assistants.
The general questionnaire provided information on sex, age, smoking, alcohol drinking and physical activity. The food frequency questionnaire assessed the habitual consumption of 178 food items and vitamin supplements during the previous year. The quantity consumed was estimated in commonly used units, household measures, or by colored photographs of foods showing different portion sizes[2]. Energy and nutrient intakes were calculated using an extended version of the 1996 computerized Dutch food composition table[15].

Blood sampling and carotenoid analyses

Non-fasting venous blood samples were obtained in sitting position in 10-mL Safety-Monovette® tubes (Sarstedt, Tilburg, The Netherlands). The samples used for preparation of plasma were collected in syringes containing 1 mL of 3.13% trisodium citrate as anti-coagulant. Filled syringes were kept at 5-10°C, protected from light. After centrifugation the next day for 20 minutes at 1,500 x g, blood fractions were aliquoted into 0.5-mL straws (CBS-IMV, l'Aigle, France) and initially frozen at -80°C before transfer into liquid nitrogen (-196°C). The 28 aliquots obtained from each subject were divided into two identical series of 14 straws. One series was stored in the local center, the other was transported to the central biorepository at IARC in Lyon, France. For the international study[12] plasma samples stored at IARC were used. For the additional sample for this study, plasma samples stored in The Netherlands were collected and transported to IARC under nitrogen in a dry shipper (CP-100, CryoPak Dry Shippers, Taylor-Wharton).

Plasma carotenoid concentrations were determined at IARC, Lyon by reversed phase high performance liquid chromatography (HPLC) following a method adapted

from Steghens et al.[16]. Seven carotenoids were analyzed: α-carotene, β-carotene, β-cryptoxanthin, lutein, lycopene, zeaxanthin and canthaxanthin. We did not study canthaxanthin separately because analysis of canthaxanthin had high between-day variation, canthaxanthin could not be determined in about half of the samples, and contribution to total carotenoids was low (0.7% on average). Values of canthaxanthin were however included in the sum of carotenoids. In the international study, between-day coefficients of variation over the entire period of analysis, i.e., 11 months, were 6.6% for α-carotene, 6.3% for β-carotene, 4.8% for β-cryptoxanthin, 7.6% for lyocpene, 6.2% for lutein, and 16.5% for zeaxanthin[12].
Cholesterol determinations were performed in the Lipid Reference Laboratory (LRL) of the University Hospital Dijkzigt in Rotterdam using standardized enzymatic methods. Total cholesterol was measured using a CHOD-PAP method (Boehringer)[17].

Statistical analysis

We associated vegetable and fruit intake assessed by the food frequency questionnaire (as independent variable) and plasma carotenoids (as dependent variables) individually, as sum of carotenoids, and combinations of individual carotenoids.
The distributions of the carotenoid plasma levels were positively skewed, therefore we used natural logarithmic transformations in the analyses, with the null values set at 10% of the lowest value (i.e., n=15 for α-carotene; n=1 for β-carotene; n=2 for lycopene; n=1 for zeaxanthin). For descriptive purposes means and standard deviations are based on non-transformed data. Correlations presented are Spearman correlations. We used SAS (version 8.1, SAS Institute, Cary, NC) for all analyses. Differences in characteristics between men and women were tested using a t-test for normally distributed variables, the Wilcoxon two sample-test for skewed data, and the Chi-square test for categorical variables. Findings were considered statistically significant if the two-sided p value was <0.05.
We used multiple linear regression (PROC GLM) to study the associations between vegetable and fruit intake (continuously) and plasma carotenoid concentrations. Moreover, we studied these relations with intake divided into quartiles. We investigated men and women separately, and explored associations at three levels: 1) the sum of vegetables, fruits and juices; 2) vegetables, fruits and juices as separate groups; 3) specific vegetables and juices, known for their (high) content of specific carotenoids. The analyses at level 3 were combined for men and women, and extended to pizza and tomato sauce. The following associations were investigated: α-carotene with intake of green/string beans, carrots, pizza and vegetable juice; β-carotene with leek, tomato, red beets, lettuce, cabbage, pepper, green/string beans, peas, spinach, endive, carrots, pizza and vegetable juice; β-

cryptoxanthin with pepper and orange/grapefruit-juice; lutein with leek, tomato, lettuce, cabbage, pepper, green/string beans, peas, spinach, endive, carrots, pizza, orange/grapefruit-juice and vegetable juice; lycopene with tomato, pepper, spinach, pizza, tomato sauce and vegetable juice; and zeaxanthin with red beets, cabbage, pepper and green/string beans. Specific fruits could not be studied individually because the intakes were highly correlated because of questionnaire design.
Associations are expressed as percentage difference in carotenoid concentration for the difference between the median intake of the lowest quartile and the median intake of the highest quartile.
We adjusted all models for potential predictors of carotenoid concentrations, i.e., age (continuously), socio-economic status (SES) (3 categories based on education), smoking status (never, ex and current), alcohol consumption (continuously), energy intake (continuously), any supplement use (yes/no), physical activity (4 categories), body mass index (BMI, kg/m^2; continuously), total plasma cholesterol (continuously), center (3 centers) and season. Analyses at level 3 were in addition adjusted for the vegetable or juice intake other than the specific types studied.
The international study showed that parameters related to the laboratory measurements and storage time were of marginal importance[11], therefore we did not adjust for these parameters.

Results

In Table 1 selected characteristics of men and women studied are presented. Women consumed more fruits and vegetables, but less energy and alcohol. The three vegetable and fruit types eaten most were the same for men and women, i.e., in descending order, cabbages, carrots and string beans/snap beans, and apples/pears, citrus fruit and bananas, respectively (data not shown).
The Spearman correlation between fruit and vegetable intake was 0.20. Fruit intake was stronger correlated with the sum of vegetable, fruit and juice intake compared to vegetable consumption, i.e., 0.80 versus 0.45.

Plasma carotenoid concentrations were higher in women than in men, except for canthaxanthin (Table 2). Lycopene contributed most to total carotenoids, followed by lutein, β-carotene, β-cryptoxanthin, α-carotene, zeaxanthin and canthaxanthin. The lycopene contribution was lower in women than in men, whereas the contributions of α-carotene and β-cryptoxanthin were higher in women (Table 2).
High correlation coefficients were found between plasma levels of α-carotene and β-carotene (0.69), α-carotene and β-cryptoxanthin (0.53), β-carotene and β-cryptoxanthin (0.54), and between lutein and zeaxanthin (0.76). Lycopene was

Table 1 Selected characteristics of the study population

	Men (n=284)		Women (n=287)	
	Mean	SD	Mean	SD
Age (years)	39.5	11.5	39.6	11.6
BMI (kg/m^2)	25.3	3.8	25.0	4.2
Socio-economic status (%)				
low	40.1		42.5	
medium	32.8		33.1	
high	27.1		24.4	
Smoking (%)				
current	38.7		37.6	
ex	30.6		26.1	
never	30.6		36.2	
Alcohol (g/d)	18.6	20.8	8.2[1]	13.8
Supplement use (%)	26.1		30.7	
Total cholesterol (mmol/L)	4.96	1.04	5.07	0.96
Energy (kJ/d)	11,394	3,411	8,371[1]	2,227
Fat intake (en%)	34.4	5.2	34.5	4.8
Vegetables (g/d)	113	49	127[1]	50
Fruits (g/d)	153	125	186[1]	145
Fruit juices (g/d)	79	92	86	89
Vegetable juices (g/d)	5	8	7	12

[1] significantly different from men (based on a t-test for normally distributed variables, the Wilcoxon two sample test for skewed data, and the Chi-square test for categorical variables)

relatively weakly correlated with the other carotenoids (0.10-0.29), whereas canthaxanthin was poorly correlated with lycopene (0.10) and not correlated with the other carotenoids. The correlation coefficients of the individual carotenoids with the total of carotenoids were all high (between 0.57-0.74), expect for canthaxanthin (0.05). Based on these correlations, combinations of α-carotene and β-carotene, α-carotene, β-carotene and β-cryptoxanthin, lutein and zeaxanthin, and of total carotenoids without lycopene and canthaxanthin were formed.

Table 2 Plasma carotenoid concentrations (μmol/L) and their contribution (%) to total carotenoid concentration

	Men (n=284)			Women (n=287)		
	Mean	SD	% of total	Mean	SD	% of total
α-Carotene	0.081	0.060	5.5	0.120	0.090	6.9[2]
β-Carotene	0.240	0.152	16.5	0.302	0.184	17.5
β-Cryptoxanthin	0.167	0.132	11.4	0.225	0.167	13.1[2]
Lutein	0.251	0.098	18.5	0.304	0.111	18.8
Lycopene	0.620	0.308	42.5	0.658	0.341	38.1[2]
Zeaxanthin	0.066	0.028	4.8	0.085	0.034	5.2
Canthaxantin	0.010	0.013	0.8	0.009	0.015	0.6
Total carotenoids[1]	1.435	0.514		1.704	0.606	

[1] sum of analyzed carotenoids; [2] significantly different from men (using the Wilcoxon two sample test)

In Table 3 correlation coefficients between plasma carotenoid concentrations (individually and in combinations), and the sum of vegetable, fruit and juice intake, and vegetables, fruits and juices separately are given. All carotenoids were positively correlated to intake, except for lycopene. Correlation coefficients ranged between -0.11 and 0.41. In general, correlations were somewhat stronger in men than in women, except for zeaxanthin. Correlations of combinations of carotenoids did not exceed the highest correlation of individual carotenoids.

Table 3 Spearman correlations between plasma carotenoid concentrations and the sum of vegetable, fruit and juice intake, and vegetables, fruits and juices as separate groups, men and women separately[1]

	Vegetables + Fruits + Juices		Vegetables		Fruits		Juices	
	♂	♀	♂	♀	♂	♀	♂	♀
α-Carotene	0.29	0.28	0.21	0.17	0.28	0.28	0.12	0.07
β-Carotene	0.24	0.17	0.19	0.15	0.25	0.18	0.09	-0.02
β-Cryptoxanthin	0.41	0.35	0.13	0.00	0.37	0.37	0.29	0.21
Lutein	0.19	0.20	0.27	0.19	0.16	0.18	0.07	0.03
Lycopene	-0.02	-0.09	0.04	0.02	-0.07	-0.11	0.04	-0.04
Zeaxanthin	0.18	0.23	0.16	0.10	0.08	0.16	0.21	0.23
Total carotenoids	0.21	0.18	0.19	0.15	0.15	0.18	0.16	0.04
Total-(lyc+cant)[2]	0.35	0.31	0.28	0.16	0.29	0.31	0.20	0.10
α-+β-carotene	0.27	0.21	0.20	0.17	0.27	0.23	0.11	-0.00
α-+β-car+β-cryp[3]	0.35	0.29	0.21	0.12	0.34	0.31	0.20	0.09
Lutein+zeaxanthin	0.20	0.22	0.26	0.18	0.14	0.19	0.10	0.08

[1] correlations from 0.12 and higher were statistically significant; [2] total carotenoids minus lycopene and cantaxanthin; [3] α-carotene plus β-carotene plus β-cryptoxanthin

In Table 4 the percentage difference in plasma concentration is given for the difference in intake between the median of the lowest vs. the highest quartile of intake. In men, plasma concentrations of α-carotene, β-carotene, β-cryptoxanthin, lutein, total carotenoids, and total carotenoids excluding lycopene and canthaxanthin were all associated with the sum of vegetable, fruit and juice intake. β-Cryptoxanthin showed the greatest percentage difference in plasma concentration. The percentage difference in concentration was higher for total carotenoids when excluding lycopene and canthaxanthin. In women but not in men the concentration zeaxanthin was associated with intake of the sum of vegetable, fruit and juice intake, while in men but not in women the concentration of β-carotene was associated. Higher intake of vegetables was marked by a higher plasma concentration of β-carotene, lutein and total carotenoids. In women, also α-carotene was associated with a higher vegetable intake. In men, β-carotene showed the largest difference between a low versus a high vegetable intake, and in women this was observed for α-carotene. A high fruit intake

was associated with higher concentrations of β-cryptoxanthin and total carotenoids. In addition, in men α-carotene and lutein concentrations were also higher. β-Cryptoxanthin concentrations differed most with fruit intake, both in men and in women. β-Cryptoxanthin and zeaxanthin concentrations were higher with higher juices intake, but only in women. Combinations of plasma carotenoids associated with intake did not show greater percentually differences between intake levels than the individual carotenoids (data not shown).

Table 4 Adjusted[1] difference in plasma carotenoids (%) between the median of the lowest and the highest quartile of intake of the sum of vegetable, fruit, juice intake, and of intake of vegetables, fruits and juices separately

	Vegetables + Fruits + Juices		Vegetables		Fruits		Juices	
	♂	♀	♂	♀	♂	♀	♂	♀
Median Q4-Q1 (g/d)	393	406	101	99	246	281	143	142
α-Carotene	35.1[2]	22.3[2]	18.1	30.0[2]	46.8[2]	11.6	-10.3	0.7
β-Carotene	20.1[2]	12.9	18.4[2]	17.0[2]	13.6	7.1	2.4	0.1
β-Cryptoxanthin	54.8[2]	41.7[2]	12.0	-8.2	48.8[2]	40.7[2]	8.8	10.7[2]
Lutein	12.9[2]	14.6[2]	15.0[2]	17.7[2]	10.8[2]	6.8	-1.1	2.1
Lycopene	-5.8	-4.6	11.4	8.9	3.7	-3.5	-12.0	-5.8
Zeaxanthin	10.9	25.0[2]	7.2	6.7	10.7	12.0	-0.6	13.9[2]
Total carotenoids	15.3[2]	14.6[2]	13.6[2]	12.4[2]	12.2[2]	10.2[2]	0.5	1.0
Total-(lyc+cant)[3]	25.3[2]	23.3[2]	15.5[2]	12.5[2]	20.2[2]	16.7[2]	2.9	4.5

[1] linear regression analyses with age, SES, smoking status, alcohol consumption, energy intake, supplement use, physical activity, BMI, total plasma cholesterol, center and season as potential confounders; [2] statistically significantly difference between the quartiles; [3] total carotenoids minus lycopene and cantaxanthin

For the significant differences shown in Table 4, the geometric means of the quartiles of intake are given in Table 5 to investigate whether plasma concentrations increased monotonously with intake or only divided low and high consumers. Around one third of the carotenoid concentrations did not increase monotonously with quartile of intake. Lutein was the only carotenoid for which concentrations increased with each quartile of vegetable intake for both men and women. For intake of fruits and juices (women only), this was the case for β-cryptoxanthin. Moreover, it was tested whether these carotenoid concentrations differed statistically across quartiles (data not shown). None of the carotenoid concentrations were statistically significantly different between all four quartiles of intake. β-Cryptoxanthin appeared to have the best discriminating power, i.e., three quartiles of the sum of vegetable, fruit and juice intake, and of fruit intake were distinguished significantly. Vegetable intake was divided only in two groups by lutein and by total carotenoids without lycopene and canthaxanthin.

Table 5 Adjusted[1] geometric means of plasma levels within the quartiles of intake (Q) of the sum of vegetable, fruit, juice intake, and of intake of vegetables, fruits and juices separately

	Q	Vegetables + Fruits + Juices		Vegetables		Fruits		Juices	
		♂	♀	♂	♀	♂	♀	♂	♀
α-Carotene	1	0.047	0.092		0.091	0.045			
	2	0.050	0.100		0.088	0.053			
	3	0.074	0.101		0.109	0.072			
	4	0.072	0.120		0.124	0.073			
β-Carotene	1	0.182		0.204	0.231				
	2	0.186		0.183	0.259				
	3	0.236		0.200	0.288				
	4	0.227		0.244	0.302				
β-Cryptoxanthin	1	0.105	0.154			0.108	0.149		0.175
	2	0.127	0.174			0.132	0.179		0.187
	3	0.163	0.209			0.159	0.196		0.194
	4	0.199	0.256			0.189	0.268		0.219
Lutein	1	0.209	0.260	0.210	0.257	0.227			
	2	0.224	0.284	0.223	0.265	0.216			
	3	0.247	0.311	0.229	0.312	0.234			
	4	0.243	0.311	0.262	0.325	0.244			
Zeaxanthin	1		0.071						0.080
	2		0.078						0.071
	3		0.079						0.083
	4		0.095						0.087
Total carotenoids	1	1.271	1.563	1.352	1.511	1.289	1.565		
	2	1.257	1.579	1.268	1.602	1.288	1.687		
	3	1.487	1.718	1.301	1.730	1.421	1.609		
	4	1.447	1.848	1.524	1.840	1.441	1.814		
Total carotenoids, without lycopene and canthaxanthin	1	0.654	0.866	0.711	0.900	0.681	0.885		
	2	0.683	0.950	0.719	0.924	0.719	0.973		
	3	0.853	1.036	0.742	1.057	0.793	0.971		
	4	0.859	1.139	0.855	1.076	0.836	1.132		

[1] linear regression analyses with age, SES, smoking status, alcohol consumption, energy intake, supplement use, physical activity, BMI, total plasma cholesterol, center and season as potential confounders

Several vegetable types and orange/grapefruit juice were associated with the plasma levels of one of the carotenoids. Results are given as percentage difference in plasma concentration for the difference in intake between the median of the lowest vs. the highest quartile of daily intake: carrot intake and α-carotene (+31% for Δ21g); intake of tomatoes and cabbage and β-carotene (+26% for Δ14g and +17% for Δ35g, respectively); intake of orange/grapefruit juice and β-cryptoxanthin (+36% for Δ112g); intake of cabbage and lutein (+13% for Δ35g); intake of tomatoes, tomato sauce and pizza and lycopene (+21% for Δ14g, +33% for Δ36g and +14% for Δ27g, respectively); and intake of cabbage and zeaxanthin (+11% for Δ35g). Geometric

means of the quartiles of intake were calculated and it was tested whether these carotenoid concentrations differed statistically across quartiles. Levels of carotenoids increased with quartile of intake, except for the associations between carrot intake and α-carotene, and pizza and lycopene. None of the carotenoid levels could distinguish all four quartiles of intake (data not shown).

Discussion

We found that plasma β-cryptoxanthin concentrations best indicated reported fruit intake. Because the variation in fruit intake contributed most to the variation in the sum of vegetable, fruit and juice intake, β-cryptoxanthin also best indicated this total intake. Although the β-carotene concentration percentually differed most between persons with low and high vegetable intake, lutein best distinguished low from high consumers. Carotenoid concentrations were however only crude markers of usual intake and could not distinguish all different quartiles of reported vegetable and fruit intake.

In 1991 and 1992 the reproducibility and relative validity of the food groups as assessed with the FFQ were tested[2]. The reproducibility after 6 months of vegetable intake was 0.80 and 0.61 for men and women respectively, and 0.76 and 0.65 after 12 months. For fruit intake, these figures were 0.70 and 0.77, and 0.61 and 0.77, respectively. The relative validity for vegetable intake, with 12 24-hour recalls as a reference, was 0.38 for men and 0.31 for women. For fruit intake these values were 0.68 and 0.56, respectively. Especially the assessment of vegetable intake appeared difficult, although our values were in the range of results of other studies[2,3]. Yet, our observed associations between individual vegetables and carotenoids, such as α-carotene with carrot intake, β-carotene and lutein with intake of cabbage, and lycopene with tomato (sauce), gave confidence in the questionnaire.

Main sources of carotenoids in the diet are vegetables and fruits. Small amounts are also found in foods of animal origin such as some fish and crustaceans, egg yolk and dairy products. Furthermore, carotenoids are taken as supplements and are used as coloring-agent in food industry.
Carotenoid levels in blood increase when persons increase their intake of vegetables, fruits and juices[18,19]. Moreover, if people are given diets with low levels of carotenoids, the plasma concentrations decrease in approximately 14-30 days and then tend to reach slowly declining plateau values[20]. Carotenoids are absorbed by duodenal mucosal cells through a mechanism involving passive diffusion and transported in the blood in lipoproteins[21]. Transport and absorption differ between carotenoids. Half-lives

of plasma β-carotene, α-carotene and β-cryptoxanthin are 7 to 14 days, of lycopene 12 to 33 days and of lutein 33-61 days[20,22]. However, fairly constant carotenoid patterns were found for up to one year[23,24]. Plasma concentrations are probably maintained from deposits in adipose tissues. It is estimated that more than 90% of the carotenoids in the body is found in tissues and <10% in plasma[25]. The concentration of β-carotene in tissues is assumed proportional to that in plasma[23]. Dietary, physiological and matrix-associated factors, such as the dietary matrix and crystalline structure of the carotenoid in the matrix, processing and cooking, nutritional status, genetic factors and the amount of carotenoids present in the diet may affect bioavailibility[21,26]. Bioavailibility of lutein from vegetables is found to be five times higher than that of β-carotene[27].

We found that plasma lutein best indicated reported vegetable intake. Lutein is widely distributed in green leafy vegetables[28]. Intervention studies showed increased plasma lutein concentrations when vegetable[29] or vegetable and fruit consumption[30,31] was enhanced. Also plasma α-carotene[29-31] and β-carotene[29-31] went up after increasing vegetable (and fruit) intakes in those studies. Observational studies mostly linked intake of carotenoids instead of vegetable and fruit consumption with plasma levels of carotenoids. The studies that did investigate vegetable intake in relation with plasma carotenoid concentrations found correlations with lutein[32-34], but also with α-carotene[32,34] and β-carotene[34], although correlation coefficients were not high. In our study plasma β-carotene showed the greatest difference between the lowest and highest vegetable intake, but appeared not to increase with each quartile of intake in men. The better performance of lutein compared to β-carotene may be a result of differences in bioavailibility or half-live times. Plasma α-carotene only indicated vegetable intake in the women in our study.
Plasma β-cryptoxanthin best marked fruit intake in our study. Citrus fruits are a major dietary source of β-cryptoxanthin in western countries[35]. In one observational study, total fruit intake was correlated with the plasma β-cryptoxanthin in men but not in women[34]. Among 20 women plasma β-cryptoxanthin was not correlated to fruit intake, while concentrations of lutein and α-carotene were[32]. Other observational studies combined fruit and vegetable intake and found the strongest correlation for plasma β-cryptoxanthin in women, while in men correlations with α-carotene and β-carotene were strongest[36]. Campbell et al.[7] included β-cryptoxanthin, and also lutein and α-carotene in their prediction equation for vegetable and fruit intake. Others found serum β-carotene positively associated with fruit and vegetable intake[37].
We are not aware of other studies that investigated the ability of plasma carotenoids to distinguish between quartiles of vegetable and fruit intake.

Based on these results it cannot be concluded that single plasma carotenoids indicate vegetable and/or fruit intake, but β-cryptoxanthin, lutein, β-carotene and α-carotene have been repeatedly related to these intakes. Part of the variance in these observations may be a result of differences in demographic, lifestyle and endogenous characteristics of the populations studied. Also differences in types of vegetables and fruits consumed, and significance of other sources of carotenoids may have played a role. In addition, also differences in measurement error in estimated vegetable and fruit intake may have accounted for this variance.

In studying plasma carotenoid concentrations as biomarkers of usual vegetable and fruit intake, we assumed a marginal contribution of dietary sources other than vegetables and fruits, and relatively stable plasma concentrations. Carotenoid concentrations may however vary during the day; concentrations measured in the morning were 6-10% higher than those in the evening[24]. Comstock et al.[38] however found ranked concentrations of carotenoids from a single sample sufficiently representative to be used as predictors of subsequent concentrations.
A greater problem for studying associations between intake and plasma levels is probably the measurement error in the intake data; this accounts mainly to the intake of vegetables. Therefore, observed associations are probably attenuated ones. The more valid measurement of fruit intake compared to vegetable intake makes it difficult to conclude whether β-cryptoxanthin better indicates fruit intake than lutein indicates intake of vegetables. In the multiple linear regression analyses, fruit intake was however the variable explaining most of the variance of the plasma β-cryptoxanthin. This was not the case for vegetable intake, i.e., the total cholesterol concentration explained most of the variance in lutein plasma levels in men and BMI in women (data not shown).

Plasma carotenoids indicated only crudely the consumption of vegetables and fruits as assessed by a FFQ. This makes plasma carotenoids for instance useful to check whether participants of an intervention study increased their intake substantially. However, plasma carotenoids may be less useful to accurately rank persons according to their usual vegetable and fruit intake.
Diminishing the measurement error of intake, for example by using repeated measurements of dietary intake, may lead to stronger associations between dietary intake and plasma levels. However, it is questionable whether such an improvement is good enough to come to a valid measurement of usual vegetable intake. Maybe a combination of dietary assessment methods and biomarkers may perform better in estimating usual intake, and subsequently helps to further elucidate the complex relation between diet and cancer[39]. Development of such methodologies deserves attention.

In conclusion, plasma carotenoid concentrations could be used to divide low from high consumers in our study population: β-cryptoxanthin for fruit intake, and lutein for vegetable intake. However, carotenoid concentrations were only crude markers of intake and were not able to distinguish between all quartiles of vegetable and fruit intake.

Acknowledgments

We would like to thank Maria van Hemert-Janssen for her excellent technical assistance with collecting and sending the blood samples to Lyon, and Annemieke van der Waal for her help with data management.

References

1. World Cancer Research Fund/American Institute for Cancer Research. Food, nutrition and the prevention of cancer: a global perspective. Washington, DC: American Institute for Cancer Research, 1997.
2. Ocké MC, Bueno-de-Mesquita HB, Goddijn HE, Jansen AM, Pols MA, Staveren van WA, Kromhout D. The Dutch EPIC food frequency questionnaire. I. Description of the questionnaire, and relative validity and reproducibility for food groups. Int J Epidemiol 1997; 26(Suppl 1): S37-S48.
3. Kaaks R, Slimani N, Riboli E. Pilot phase studies on the accuracy of dietary intake measurements in the EPIC Project: Overall evaluation of results. Int J Epidemiol 1997; 26(Suppl 1): S26-S36.
4. Willett W, Lenart E. Reproducibility and validity of food-frequency questionnaires. In: Nutritional epidemiology. Second Edition. W Willett. New York/Oxford: Oxford University Press, 1998: 101-147.
5. Kaaks R, Riboli E, Sinha R. Biochemical markers of dietary intake. In: Toniolo P, Boffetta P, Shuker DEG, Rothman N, Hulka B, Pearce N (Eds.). Application of biomarkers in cancer epidemiology. Lyon, France: IARC, 1997: 103-126.
6. Virtanen SM, Veer P van 't, Kok F, Kardinaal AF, Aro A. Predictors of adipose tissue carotenoid and retinol levels in nine countries. The EURAMIC Study. Am J Epidemiol 1996; 144: 968-979.
7. Campbell DR, Gross MD, Martini MC, Grandits GA, Slavin JL, Potter JD. Plasma carotenoids as biomarkers of vegetable and fruit intake. Cancer Epidemiol Biomarkers Prev 1994; 3: 493-500.
8. Veer P van 't, Kardinaal AF, Bausch-Goldbohm RA, Kok FJ. Biomarkers for validation. Eur J Clin Nutr 1993; 47(Suppl 2): S58-S63.
9. Brady WE, Mares-Perlman JA, Bowen P, Stacewicz-Sapuntzakis M. Human serum carotenoid concentrations are related to physiologic and lifestyle factors. J Nutr 1996; 126: 129-37.

10. Olmedilla B, Granado F, Blanco I, Rojas-Hidalgo E. Seasonal and sex-related variations in six serum concentrations, retinol, and alpha-tocopherol. Am J Clin Nutr 1994; 60: 106-110.
11. Riboli E, Kaaks R. The EPIC Project: rationale and study design. European Prospective Investigation into Cancer and Nutrition. Int J Epidemiol 1997; 26(Suppl 1): S6-S14.
12. Kappel AL van, Ferrari P, Slimani N, Steghens J-P, Johansson I, Wallström P et al. Plasma concentrations of 6 carotenoids in 9 European countries: report from the European Prospective Investigation into Cancer and Nutrition (EPIC). Submitted.
13. Smit HA, Verschuren WMM, Bueno-de-Mesquita HB, Seidell JC. Monitoring van risicofactoren en gezondheid in Nederland (MORGEN-project): Doelstelling en werkwijze (in Dutch). Report number 263200001. Bilthoven, The Netherlands: RIVM, 1994.
14. Loon AJM van, Tijhuis M, Picavet HSJ, Surtees PG, Ormel J. Survey non-response in The Netherlands: effects on prevalence estimates and associations. Submitted.
15. Stichting NEVO. NEVO tabel. Nederlands Voedingsstoffenbestand 1996 (in Dutch). Den Haag, The Netherlands: Voorlichtingsbureau voor de Voeding, 1996.
16. Steghens JP, van Kappel AL, Riboli E, Collombel C. Simultaneous measurement of seven carotenoids, retinol and alpha-tocopherol in serum by high-performance liquid chromatography. J Chromatogr B Biomed Sci Appl 1997; 694: 71-81.
17. Katterman R, Jaworek D, Möller G. Multicenter study of a new enzymatic method of cholestrol determination. J Clin Chem Clin Biochem 1984; 22: 245-251.
18. Zino S, Skeaff M, Williams S, Mann J. Randomised controlled trial of effect of fruit and vegetable consumption on plasma concentrations of lipids and antioxidants. BMJ 1997; 314: 1787-1791.
19. Broekmans WM, Klopping-Ketelaars IA, Schuurman CR, Verhagen H, Berg H van den, Kok FJ, Poppel G van. Fruits and vegetables increase plasma carotenoids and vitamins and decrease homocysteine in humans. J Nutr 2000; 130: 1578-1583.
20. Rock CL, Swendseid ME, Jacob RA, McKee RW. Plasma carotenoid levels in human subjects fed a low carotenoid diet. J Nutr 1992; 122: 96-100.
21. Parker RS. Aborption, metabolism, and transport of carotenoids. FASEB J 1996; 10: 542-551.
22. Micozzi MS, Brown ED, Edwards BK, Bieri JG, Taylor PR, Khachik F, Beecher GR, Smith Jr. JC. Plasma carotenoid response to chronic intake of selected foods and β-carotene supplements in men. Am J Clin Nutr 1992; 55: 1120-1125.
23. Peng Y-M, Peng Y-S, Lin Y, Moon T, Roe DJ, Ritenbaugh C. Concentration and plasma-tissue-diet relationships of carotenoids, retinoids and tocopherols in humans. Nutr Cancer 1995; 23: 233-246.
24. Cantilena LR, Stukel TA, Greenberg ER, Nann S, Nierenberg DW. Diurnal and seasonal variation of five carotenoids measured in human serum. Am J Clin Nutr 1992; 55: 659-663.
25. Bendich A, Olsen JA. Biological actions of carotenoids. FASEB J 1989; 3: 1927-1932.
26. Pee S de, West CE. Dietary carotenoids and their role in combating vitamin A deficiency: a review of the literature. Eur J Clin Nutr 1996; 50(Suppl 3); S38-S53.

27. Hof KH van het, Brouwer IA, West CE, Haddeman E, Steegers-Theunissen RPM, Dusseldorp M van et al. Bioavailibility of lutein from vegetables is five times higher than that of β-carotene. Am J Clin Nutr 1999; 70: 261-268.
28. Ong ASH, Tee ES. Natural source of carotenoids from plants and oils. Meth Enzymol 1992; 213: 142-167.
29. Martini MC, Campbell DR, Gross MD, Grandits GA, Potter JD, Slavin JL. Plasma carotenoids as biomarkers of vegetable intake: the University of Minnesota Cancer Prevention Research Unit feeding studies. Cancer Epidemiol Biomarkers Prev 1995; 4: 491-496.
30. LeMarchand L, Hankin JH, Scott Carter F, Essling C, Luffey D, Franke AA et al. A pilot study on the use of plasma carotenoids and ascorbic acid as markers of compliance to a high fruit and vegetable dietary intervention. Cancer Epidemiol Biomarkers Prev 1994; 3: 245-250.
31. Smith-Warner SA, Elmer PJ, Tharp TM, Fosdick L, Randall B, Gross M et al. Increasing vegetable and fruit intake: randomized intervention and monitoring in an at-risk population. Cancer Epidemiol Biomarkers Prev 2000; 9: 307-317.
32. Polsinelli ML, Rock CL, Ahlstrom Henderson S, Drewnowski A. Plasma carotenoids as biomarkers of fruit and vegetable servings in women. J Am Diet Assoc 1998; 98: 194-196.
33. Thurnham DI, Northrop-Clewes CA, Chopra M. Biomarkers of vegetable and fruit intakes. Am J Clin Nutr 1998; 68: 756-757.
34. Michaud DS, Giovannucci EL, Ascherio A, Rimm EB, Forman MR, Sampson L, Willett WC. Associations of plasma carotenoid concentrations and dietary intake of specific carotenoids in samples of two prospective cohort studies using a new carotenoid database. Cancer Epidemiol Biomarkers Prev 1998; 7: 283-290.
35. Mangels AR, Holden JM, Beecher GR, Forman MR, Lanza E. Carotenoid content of fruits and vegetables: An evaluation of analytic data. J Am Diet Assoc 1993; 93: 284-296.
36. Tucker KL, Chen H, Vogel S, Wilson PWF, Schaefer EJ, Lammi-Keefe CJ. Carotenoid intakes, assessed by dietary questionnaire, are associated with plasma carotenoid concentrations in and elderly population. J Nutr 1999; 129: 438-445.
37. Drewnowski A, Rock CL, Henderson SA, Shore A, Fischler C, Galan P et al. Serum β-carotene and vitamin C as biomarkers of vegetable and fruit intakes in a community-based sample of French adults. Am J Clin Nutr 1997; 65: 1796-1802.
38. Comstock GW, Burke AE, Hoffman SC, Norkus EP, Gross M, Helzlsouer KJ. The repeatability of serum carotenoid, retinoid, and tocopherol concentrations in specimens of blood collected 15 years apart. Cancer Epidemiol Biomarkers Prev 2001; 10: 65-68.
39. Toniolo P, Akhmedkhanov A. Toniolo and Akhmedkhanov respond to "serum carotenoids and breast cancer" by Rohan. Am J Epidemiol 2001; 153: 1151.

Chapter 8

Modeling the potential reduction in cancer incidence: the case of fruits and vegetables in The Netherlands

Margje CJF Jansen, Karin FAM Hulshof, Rudolf T Hoogenveen, WM Monique Verschuren, Daan Kromhout, Frans J Kok

Submitted

Abstract

Fruits and vegetables have been consistently inversely associated with cancer risk. Consumption of these foods is below recommended daily intake in many Western populations, including The Netherlands. We described the fruit and vegetable consumption and the change in this consumption over a 10 year-period, i.e., 1987/88-1997/98, for the Dutch population aged 19 years and older using data of the National Food Consumption Surveys from 1987/88 (n=4,134), 1992 (n=4,466) and 1997/98 (n=4,661).

Based on the evidence from observational epidemiological studies, we estimated which part of the cancer incidence would be potentially preventable over a 40-year period by increasing the current fruit and vegetable intake using a computer simulation model. We did this for a subgroup of the population only, i.e., persons 19-35 years of age with a low level of education, because they consumed the lowest amounts of fruits and vegetables.

In 1997/98, mean fruit and vegetable intakes were 105 g/d and 142 g/d in men, and 122 g/d and 138 g/d in women, respectively. We estimated that if all persons aged 19-35 with a low level of education would adhere to the dietary guideline of 400 grams of fruits and vegetables daily, 14 to 22% of the cancer incidence would potentially be prevented over a 40-year period. The other, more realistic, scenarios simulated resulted in smaller reductions, i.e., in the range of 1-7%.

In conclusion, based on current evidence, enhancing fruit and vegetable intake to the level of the dietary guideline may lead to a potential reduction in cancer incidence of 14 to 22%. However, this ideal situation may not be realistic, and therefore smaller reductions should be expected in reality.

Introduction

Despite the postulated beneficial effects of fruits and vegetables on cancer risk[1], results from the Dutch National Food Consumption Surveys show that the intake of these foods is below the recommended daily level of 200 grams fruits and 150-200 grams vegetables in the majority of the adult population. Furthermore, these surveys show that the consumption has declined during the 10-year period 1987/1988-1997/1998[2]. Also in other countries, such as the United States[3] and northwestern European countries such as the United Kingdom[4], Ireland[5] and Denmark[6], a substantial proportion of the population does not comply with dietary guidelines for fruit and vegetable intake.

Besides time trends in fruit and vegetable consumption, also socio-demographic variables, such as sex, age and educational level, have been related to the amount

of these plant foods eaten, although these relations may not be equal in all societies. Moreover, certain lifestyle characteristics have been associated with level of fruit and vegetable consumption, e.g. smokers consuming less fruits[7].

In the present paper, we describe the level of fruit and vegetable consumption and the changes in this consumption over a 10 year-period, i.e., 1987/88-1997/98, for the Dutch population aged 19 years and older. Based on these figures, we identified a subgroup of special interest for studying the potential impact of enhancing fruit and vegetable consumption on cancer incidence. This subgroup consists of persons aged 19-35 years with a low level of education. They eat the lowest amounts of fruits and vegetables and are young enough, considering the latency time of cancer of several decades, to be of interest for prevention strategies. We used a computer simulation model to estimate the potential reduction in cancer incidence over a 40-year period by increasing the fruit and vegetable intake according to different scenarios.

Methods

Fruit and vegetable intake

Data on fruit and vegetable consumption in The Netherlands are based on the three Dutch National Food Consumption Surveys carried out in 1987/88, 1992 and 1997/98, respectively. The numbers of participants were 5,898 in 1987/88, 6,218 in 1992 and 6,250 in 1997/98 with response rates of 79.5%, 71.5%[2] and 68.5%[8], respectively. From an existing panel, households were selected and individual data were collected from the household members aged 1 year and older. The panel consisted of a stratified probability sample of non-institutionalized households in The Netherlands. In the first two surveys, households with a head housekeeper aged 75 or over were excluded. In the last survey this exclusion criterion was abandoned. Socio-demographic characteristics of the panel members could be obtained from the panel holder, i.e., the marketing research institute GFK.

A two-day weighed dietary record-method was used to assess dietary intake. Trained dietitians instructed the participants for recording consumption data and assessed volume measures. The survey was distributed equally over the seven days of the week and over a whole year, whereas holiday periods were excluded. For each individual the average intake over two days was calculated, and the food products were categorized into 23 major food groups, among which fruits and vegetables. In the food groups 'fruits' and 'vegetables', potatoes, legumes and nuts were not included[9].

We calculated the intake per survey for men, women, sex-age groups and according to educational level for those 19 years and over. Pregnant women (1987/88, n=52; 1992, n=58; 1997/98, n=50) were excluded, leaving data of 4,134, 4,466 and 4,661 persons from the three successive surveys, respectively. Intake of fruit juices was calculated separately. We used the following age groups: 19-35, 36-49, 50-64 and 65 years and over. A low level of education was defined as primary school, lower vocational or lower general education as highest level of completed education, whereas a high education was defined as completed higher vocational or university education.
For the descriptive analyses, we used SAS (version 8.1, SAS Institute, Cary, NC). The Kruskall-Wallis test was used to compare dietary intake across categories. The significance level was set at 0.05.

Estimating health gain

We estimated the potential reduction in cancer incidence over a 40-year period following several scenarios for increasing fruit and vegetable intake. These estimates were restricted to persons aged 19-35 years with a low level of education. In The Netherlands, this group consists of around 1,000,000 persons.
We used the Chronic Diseases Model of the National Institute of Public Health and the Environment for estimating this reduction in cancer incidence[10]. In this computer model trends or interventions regarding socio-demographic or lifestyle factors can be simulated and subsequent health effects can be estimated. The relation between risk factors and chronic diseases is build in, in terms of relative risks. These relative risks are deduced from observational epidemiological studies and intervention studies available in literature.
The model is based on the life-table method and subsequently extended to a multi-state transition model[11]. The general idea is that each person belongs to a risk factor class, in our situation fruit and vegetable intake. For each risk factor class a relative risk for the disease under study is defined, in our case cancer incidence. Based on the prevalence distribution of a population and the accompanying risk estimates, cancer incidence is estimated using the computer model. Following scenarios specified, another prevalence distribution of the risk factor is calculated and used for estimating subsequent cancer incidence. Thus, during simulation some persons become diseased; in our simulation they become an incident cancer case. Moreover, by including national data of the two major causes of death, i.e., cancer and coronary heart disease, in the model, persons may die in the simulation. Diseased persons remain at risk for getting other diseases. This approach results in a dynamic population. In our calculation we simulated for 40 years and calculated the cumulative number of new cancer cases over this period. We chose a period of

40 years because this is a relevant time frame in carcinogenesis for the studied age group.

We derived information on fruit and vegetable consumption from the Dutch National Food Consumption Survey of 1997/98. In order to come to an estimation of the fruit and vegetable intake representative for the whole Dutch population in this age range with a low educational level, weighing factors delivered by the marketing research institute GFK were used.
We categorized fruit and vegetable consumption by dividing the combined intake into five categories, i.e., less than 100, 100-199, 200-299, 300-399 and 400 g/d or more. Intake of fruits is considered both with and without intake of fruit juices. The highest consumption category stands for those adhering to the recommended intake of at least 400 grams per day, i.e., 200 grams of fruit and 200 grams of vegetables. We assumed that this highest category corresponds with the highest potential risk reduction of cancer (lowest RR) and the lowest consumption category with no risk reduction (RR=1, reference category). For the categories in between, risk reductions were log-linearly interpolated. The relative risks used were extracted from the vast amount of epidemiological studies performed in this area as summarized by Klerk et al.[12]. They gave three estimates of 'the' relative risk per cancer site, i.e., a conservative, a best guess, and an optimistic value. For this analysis, we used the conservative and the best-guess estimates (given in the Appendix). Relative risks were assumed to be independent of sex, age and education.

We simulated the effect of four different scenarios for increasing the fruit and vegetable consumption after 1997/98. The 'effect' has been defined as the difference in cancer incidence between the reference scenario and an alternative scenario. We expressed results both in absolute numbers and in relative numbers. We analyzed men and women separately, and aggregated over the sexes. The following scenarios have been simulated: **Reference scenario**: consumption remains the same as in 1997/98; **Trend scenario**: consumption returns to the level of 1987/88, thus, counteracting the unfavorable decline over the past 10 years; **Guideline scenario**: consumption is according to the dietary guideline of at least 400 grams of fruits and vegetables per day, i.e., the most ideal situation; **Education scenario**: consumption reaches the more favorable level of the highly-educated in the same age range, thus, counteracting the unfavorable effect of a low educational level; **Age scenario**: consumption reaches the level of the 50-65 year olds with the same (low) educational level, thus, counteracting the unfavorable situation in the young versus the old.

We assumed an inverse relationship with fruit and vegetable intake for the following cancer types: cancers of the oral cavity, larynx, esophagus, lung, stomach, colon, rectum, bladder, kidney, pancreas, breast, endometrium, cervix, ovary and prostate. We applied the simulation model to each cancer type separately, but reported total cancer by summing up the incidence numbers per cancer. The cancer types considered contributed 72% to the total cancer incidence and 76% to the total cancer mortality in The Netherlands in 1994. The remaining types of cancer were not included in the computer model. Moreover, we reported the three cancer types that are potentially most preventable per sex.

We used Dutch population composition data[13], disease-specific and total mortality data[14] from Statistics Netherlands. Cancer incidence data specified to sex and 5-years age categories came from the Netherlands Cancer Registry[15].

Results

In Table 1 the mean and standard deviation of the intake of vegetables, fruits, fruits including fruit juices, vegetables and fruits, and vegetables, fruits and fruit juices are given for men and women separately per survey. The mean intakes were significantly lower in 1997/98 than in 1987/88, except for the intakes including fruit juices. The largest contrasts were observed between 1987/88 and 1992. Intakes between 1992 and 1997/98 did not differ significantly. Men consumed more vegetables than women in 1987/88 and 1992, whereas the difference in 1997/98 was not statistically significant. Women ate more fruits in all three surveys.

Table 1 Fruit and vegetable intake (g/d) and age (years) (mean ± SD) in the three Dutch National Food Consumption Surveys for men and women aged 19 years and older

	1987/88		1992		1997/98	
	Men (n=1,930)	Women (n=2,204)	Men (n=2,058)	Women (n=2,408)	Men (n=2,117)	Women (n=2,544)
Age	42.7 ± 15.1	42.8 ± 15.4	43.1 ± 14.9	42.9 ± 15.1	44.4 ± 15.9	45.8 ± 16.9
Vegetables	159 ± 104	150 ± 98	147 ± 100	140 ± 95	142 ± 102	138 ± 96
Fruits	122 ± 132	133 ± 124	112 ± 126	126 ± 125	105 ± 122	122 ± 121
Fruits and juices	153 ± 156	187 ± 163	151 ± 166	191 ± 177	157 ± 174	201 ± 188
Vegetables and fruits	281 ± 183	283 ± 168	259 ± 172	266 ± 169	247 ± 173	260 ± 170
Vegetables, fruits and juices	313 ± 203	337 ± 198	299 ± 205	330 ± 211	299 ± 212	339 ± 223

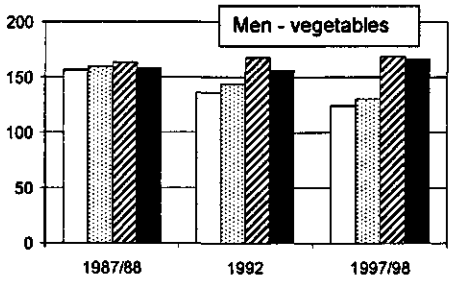


Figure 1A

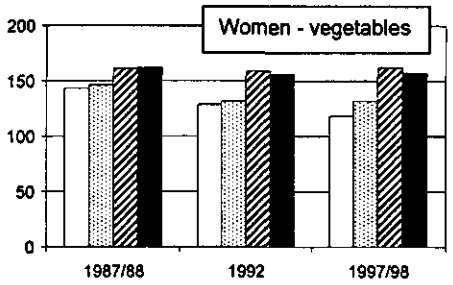


Figure 1B

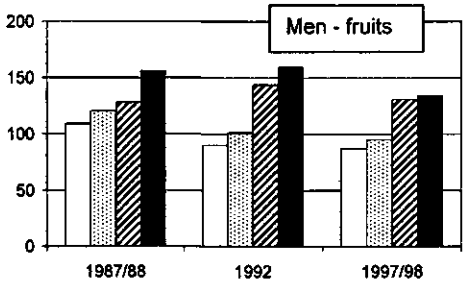


Figure 1C

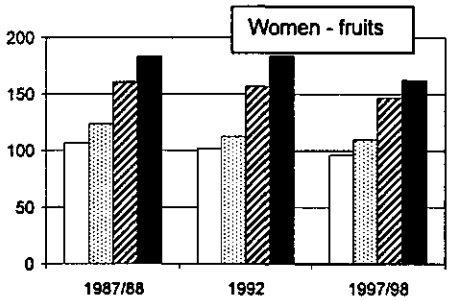


Figure 1D

Age groups □ 19-35; ⊡ 36-49; ▨ 50-64; ■ 65+

Figure 1 Mean vegetable intake (g/d) in the three successive surveys per age-group in men (A) and women (B); and mean fruit intake (g/d) in these surveys per age-group in men (C) and women (D)

Mean vegetable consumption in men (Figure 1A) and in women (Figure 1B) was lowest in the youngest age group, i.e., 19-35 years, in each survey except for men in 1987/88. Also fruit consumption was lowest in the youngest men (Figure 1C) and women (Figure 1D). Although mean intakes of vegetables and fruits decreased during the 10-year period, figures 1A-1D show that this decline was mainly seen in the younger age groups. Vegetable intake did not decrease in those 50 years of age and over. Fruit intake, however, was lower in 1997/98 in men and women 65 years and older compared to the consumption in the other surveys.

In Figures 2A-2D mean intakes of vegetables and fruits by educational level are shown for men and women aged 19-35 years in all three surveys. In general, intakes were lowest in those with a low level of education. The difference between low and high educational level was most pronounced for fruit consumption in women.

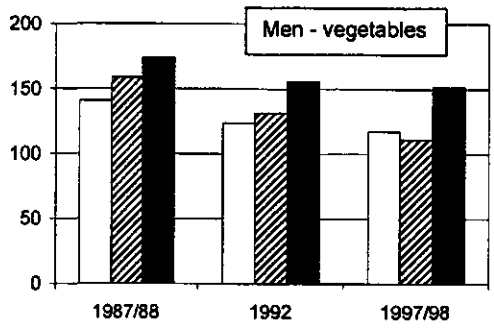


Figure 2A

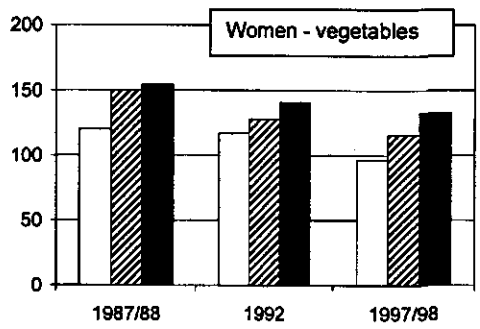


Figure 2B

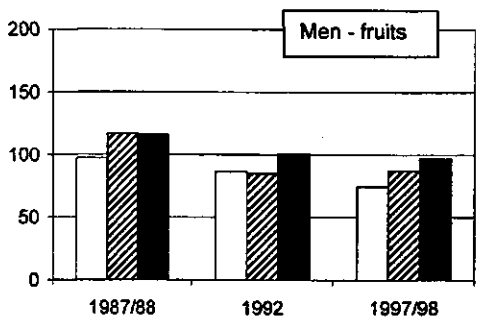


Figure 2C

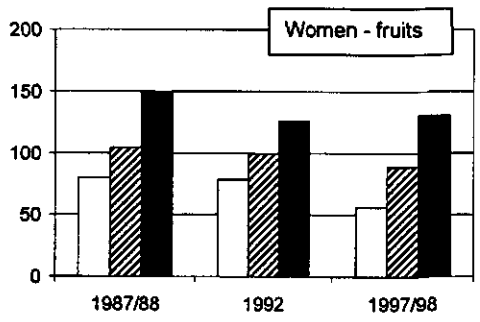


Figure 2D

Eductional levels □ low; ▨ medium; ■ high

Figure 2 Mean vegetable intake (g/d) in the three successive surveys per educational level in persons 19-35 years of age for men (A) and women (B); and mean fruit intake (g/d) in these surveys per educational level in this age-group for men (C) and women (D)

Based on these results the subgroup of persons 19-35 years of age with a low level of education was identified as risk group. For this group, we performed the simulation study to estimate the potential reduction in cancer incidence over a period of 40 years.

As described in the Method section, the risk factor we simulated was fruit and vegetable intake combined, divided into five categories. In Table 2 the prevalences in these five categories for the situation excluding fruit juices per scenario are given. The reference scenario describes the situation in 1997/98. From this table it is clear that only a small percentage of the group studied, i.e., persons aged 19-35 with a low level of education, met the dietary guideline for fruit and vegetable consumption.

Compared to the distribution of the trend scenario, i.e., the situation of 1987/88, this picture has deteriorated. Moreover, by comparing the reference scenario with the education and age scenarios, it seems that adherence to the dietary guideline in the studied group was less favorable than in those of the same age but with high education, or in those with a low level of education but in the age range of 50-65. A similar distribution in these five categories was made for the intake including fruit juices (data not shown).

Table 2 Percentage of men and women aged 19-35 years with a low level of education in the five categories of fruit and vegetable in the different scenarios used for modeling

	Fruit and vegetable consumption[1] (g/d)				
	< 100	100-199	200-299	300-399	400+
Reference scenario					
men	24.6	33.7	19.1	12.9	9.8
women	37.5	32.0	20.2	8.6	1.6
Trend scenario					
men	19.7	29.7	19.4	15.7	15.6
women	20.7	34.3	23.8	12.7	8.5
Guideline scenario					
men	0	0	0	0	100
women	0	0	0	0	100
Education scenario					
men	18.0	27.8	24.1	15.5	14.6
women	15.1	24.5	24.1	21.1	15.2
Age scenario					
men	17.1	22.5	21.5	20.5	18.3
women	13.7	21.6	22.8	18.4	23.5

[1] excluding fruit juices

We calculated the expected cancer incidence over a 40-year period in the reference scenario for fruit and vegetable consumption. The cumulative absolute numbers were around 70,000 cancer cases for men and around 50,000 for women in a population of 1,000,000 persons at risk. Similar simulations were done for the alternative scenarios. The differences between the reference and alternative scenarios are depicted in Figure 3 as percentages of cancer cases that could be prevented for men and women combined both using the 'best guess' and the 'conservative' estimates of the relative risks. In Table 3 the accompanying number of incident cancer cases are given for men and women separately and combined.

As expected, the guideline scenario provided the highest potential cancer reduction, i.e., in the range of 15-22% (18,386-27,955 cases). The health gain estimated according to the education scenario and the age scenario were both lower than in the guideline scenario, but in the same range, i.e., 3-6% (3,416-7,779 cases). The trend scenario was estimated to provide the least health gain, i.e., from 2 to 3% of the cancer cases (2,459-3,914 cases). Logically, the estimates using the

conservative relative risk values were consequently lower than those using the best guess values.

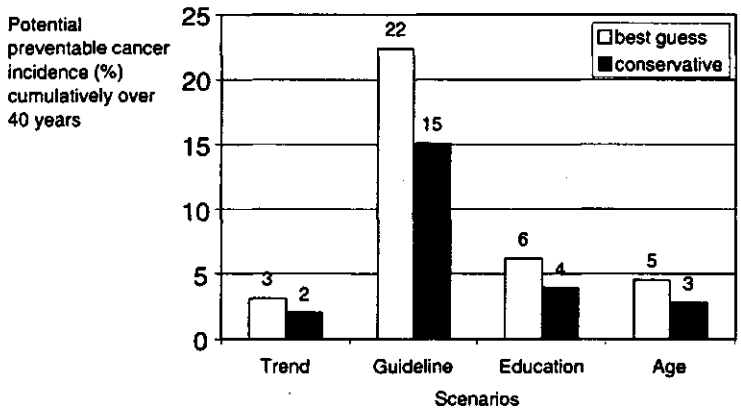


Figure 3 Potential reduction in cancer incidence (%) cumulatively over 40 years for the scenarios 'trend', 'guideline', 'education' and 'age' compared to the reference scenario for men and women 19-35 years with a low level of education using the best guess and the conservative value of the relative risk

Table 3 Cumulative number of incident cancer cases potentially preventable by enhancing fruit[1] and vegetable intake according to the scenarios[2] 'trend', 'guideline', 'education' and 'age' for men and women 19-35 years with a low level of education using the best guess and conservative ('cons') relative risk

	Number of incident cancer cases potentially prevented					
	Men		Women		Total	
	best guess	cons	best guess	cons	best guess	cons
Trend scenario	2,140	1,586	1,774	873	3,914	2,459
Guideline scenario	17,990	13,460	9,965	4,926	27,955	18,386
Education scenario	3,866	2,864	3,913	1,930	7,779	4,794
Age scenario	2,453	1,809	3,263	1,607	5,716	3,416

[1] excluding fruit juices; [2] Trend: consumption returns to the level of 1987/88; Guideline: consumption is according to the dietary guideline of at least 400 grams of fruits and vegetables per day; Education: consumption reaches the more favorable level of the highly-educated in the same age range; Age: consumption reaches the level of the 50-65 year olds with the same (low) educational level; see also Method-section

Sex-specific results, based on the best guess estimate for the RR were 3% for both men and women in the trend scenario; 25% for men and 19% for women in the guideline scenario; 5% for men and 7% for women in the education scenario; and 3% for men and 6% for women in the age scenario. We repeated the analyses including fruit juices as fruit intake. Estimates of the potential preventable incident cases were 14-20%, 3-5%, 4-7% and 1-2% for the scenarios guideline, education, age and trend, respectively. All estimates were somewhat lower, except for the age scenario.
Combining results using the best guess and conservative values of the risk estimates and the scenarios in- and excluding fruit juices leads to the maximum theoretically cancer reduction of 14-22%.

The three cancers contributing mostly to a decrease in cancer incidence by enhancing the fruit and vegetable consumption were in descending order for men lung cancer (41%), colon cancer (11%) and stomach cancer (10%), and for women breast cancer (31%), lung cancer (18%) and colon cancer (13%).

Discussion

Fruit and vegetable consumption

Comparing mean fruit and vegetable consumption of adults in The Netherlands with those in other countries is hindered by differences in assessment methods used, in classifications of fruit and vegetable intake, and over time. However, intakes (g/d) in several countries are given: Denmark: vegetables (including potatoes) 255 in men and 205 in women, fruits (including juices) 152 in men and 179 in women[6]; Finland: fruits, berries and vegetables 433[16]; Sweden: fruits and vegetables 240 in men and 290 in women[17]; Germany: fruits and vegetables (without processed fruits and vegetables) 338 in men from East-Germany and 231 from West-Germany, and 359 in women from East-Germany and 256 from West-Germany[18]; Ireland, fruits and vegetables 199[5]; United Kingdom: fruits and vegetables, 253 in men and 242 in women[4]; France: vegetables 93 in men and 109 in women, fruits 189 in men and 184 in women[19]; Italy: fruits and vegetables 433[20]; Spain: fruits and vegetables in men 455 and 500 in women[21]; and United States: 4.3 servings/day (estimated to be 344 g)[3]. In Europe, there seems to be a north-south gradient, with high levels of intake in the south. Intakes in countries geographically situated around The Netherlands are roughly in the same range.

Simulating health gain

As expected, the potential health gain was greatest in the guideline scenario. In our simulation study we saw that the differences in fruit and vegetable intake within the population (according to age and educational level) were associated with greater differences in the expected cancer incidence than the decline in fruit and vegetable consumption over the 10-year period.

The potential preventable effect seemed greater for men than for women following the guideline scenario, i.e., 23-25% versus 16-19%. This is mainly due to higher lung cancer incidence in men than in women. Fruit and vegetable consumption differed more with age and educational level in women than in men. This resulted in higher percentages of reduced cancer incidence in women than in men in the education and age scenarios. The results in relative numbers for the trend scenario were in the same order of magnitude for men and women.

Methodological considerations

The consumption surveys in The Netherlands were carefully designed for assessing dietary intake. The method used estimates the mean intake in a valid way on group-level, the variation in intake is however less accurately assessed due to the use of two consecutive days. Other weaknesses of these consumption data may lie in the fact that selection may have occurred while constructing the panel, and that not all data were independent because subjects could come from the same households.

For the purpose of modeling, we had to make major simplifications relevant to the validity of the forecasts. For example, we divided the 19-35 year olds into five discrete consumption groups. However in reality, consumption is distributed continuously. Moreover, relative risks used are estimates for the general population and not specifically applicable for the group studied. However, we are not aware of age-specific relative risks for the associations studied. Another simplification is that besides fruit and vegetable intake other factors affect cancer risk, which we assumed constant in our analyses. We also assumed no interaction with other risk factors; for example, smokers experiencing a greater reduction in cancer incidence than nonsmokers. Another aspect is that there is a latency period of several decades between changes in lifestyle and cancer incidence. In our simulation model, we did not build in a lag-time to take into account this latency period. However, by simulating over 40 years we imitated a sort of latency period. Forty years as simulation period is an arbitrary choice. On the one hand a long period is required because of the long induction and latency period of cancer and consequently the largest part of cancer incidence occurs later in life. On the other hand all data used and assumptions made are based on current data and

knowledge. The longer the period of simulation, the greater the chance that these data and assumptions are less applicable. For example, life expectancy and case-fatality are not static but change over time. Although the 40 years chosen is a long period, a large proportion of the cancer cases occurs even later in life, i.e., when people are older than 59-75 years[15].

Etiological considerations

Associations between fruit and vegetable consumption and cancer risk have been reviewed by many[1,22-25]. Moreover, already in 1981 Doll and Peto[26] estimated the role of dietary factors in cancer etiology at 35% with a wide confidence range of 10-70%. In 1995, Willett came to an estimation of 32% with a range of 20-42%[27]. However, many aspects of the potential protective effect of fruits and vegetables are still unclear. Based on the available literature we cannot conclude that associations consistently differ between vegetables and fruits. In some studies stronger relationships for fruits were found, while in other studies for vegetables; and these results also varied by cancer site[1]. Therefore, we assumed equal inverse associations for fruits and vegetables, and used the combined fruit and vegetable intake in our model. Moreover, there is no international consensus whether intake of fruit juices should (fully) contribute to fruit intake when studying the relations between intake and cancer risk. Also for potatoes and legumes inclusion in vegetable intake is not universal around the world. Regarding fruit juices, we chose to perform the simulations both with and without fruit juices included as fruit. Because potatoes and pulses are not counted as vegetables in dietary practices in The Netherlands, we left them out. Moreover, evidence for a cancer-preventing effect of potatoes and pulses is very limited. These differences in used definitions for fruits and vegetables lead to uncertainties in risk estimates.

The strength of associations between fruit and vegetable consumption and cancer types varies widely in literature and is still subject for debate. Because of this uncertainty we used two estimates for the relative risks in our simulation study.

Also the duration of a specific level of fruit and vegetable consumption necessary for a decrease in cancer risk is unknown, as is the existence of critical periods in life in cancer development.

Other simulation studies

Also Klerk et al.[12] estimated the potential preventable cancer incidence through enhancing the fruit and vegetable intake to the recommended level for the Dutch situation. They used the population attributable risk (PAR) for this purpose. Moreover, fixed population composition data were used and the estimation had no time dimension. The report estimated cancer prevention to be 7 to 28%, with 19% as best guess. In our simulation we used the same conservative and best guess

relative risks, and came to an estimate of 14 to 22% and added a time horizon of 40 years. Our estimation was therefore somewhat higher compared with their 7-19%. Probably this is mainly the result of the fact that we did not include total cancer, and that the mix of most prevalent cancers differs with age and that we studied a relatively young population.
In another Dutch report[28], two policy interventions regarding fruit and vegetable intake were simulated in the computer model PREVENT for their impact on cancer incidence. First a 'fruit and vegetable-program' at primary schools, and second an educational campaign to stimulate fruit and vegetable intake in the whole Dutch population. They simulated over 50 years and estimated the number preventable deaths due to lung cancer, stomach cancer, colon cancer and bladder cancer. Numbers were reported per cancer site for men and women separately per year, but no attempt was made to report a cumulative figure. However, their calculated preventable cancer deaths were much lower than our figures for incidence. Major reasons for this difference are the limited types of cancers included, less extreme interventions simulated, estimating cancer deaths instead of cancer incidence, and the intervention in primary school children did not yield substantial effects on cancer incidence within 50 years due to the low age of the group studied.
We did not found international studies that used comparable computer simulations to estimate the potential preventable cancer incidence by fruit and vegetable intake.

Theoretically versus practical achievable health gain

We calculated health gain that is theoretically achievable and did not deal with the practical feasibility of the different scenarios. We know that it is difficult to enhance fruit and vegetable intake in the long term. Intervention/promotion programs mostly achieve a rise of around half a serving per day, i.e., around 40 grams[29,30]; sometimes somewhat higher up to around one serving[31,32]. Therefore, the guideline scenario leading to the reduction of 14-22% of cancer incidence may not be realistic. The other scenarios simulated may reflect changes in fruit and vegetable intake that are practical achievable. Thus, expected reductions in cancer incidence in reality will be lower than the estimated maximum of 14-22% of cancer incidence.

Conclusion

We showed that the mean fruit and vegetable consumption in The Netherlands is below the recommended intake. Consumption was lowest in 19-35 year olds with a low level of education. Given evidence from observational epidemiological studies, we estimated that increasing fruit and vegetable consumption to the intake level recommended by the present dietary guidelines, could potentially decrease cancer incidence by 14 to 22% over a 40-year period. The other, more realistic, scenarios simulated resulted in smaller cancer reductions, i.e., in the range of 1-7%. Although

the used methodology has its limitations, to our opinion the results give a good picture of the extent of gain in public health theoretically possible given current knowledge. Our approach gives the opportunity to compare different (public health) scenarios on their impact on disease incidence, and enables to include population dynamics and the time dimension. Moreover, such a systematic approach reveals the gaps in our knowledge concerning the relation between fruit and vegetable intake and cancer risk. If we gain further insight in this association, we can refine the simulations leading to better estimations of the expected cancer incidence.

Acknowledgments

Under the authority of the Commission 'Trends Food Consumption' of the Dutch Health Council and financed by the Ministry of Health, Welfare and Sport, among others health effects following changes in fruit and vegetable consumption were estimated in the way presented in this paper[33]. We want to thank W Bosman MSc of the Dutch Health Council for his intellectual input into this work. Moreover, we want to thank Dr MC Ocké for her useful comments.

References

1. World Cancer Research Fund/American Institute for Cancer Research. Food, nutrition and the prevention of cancer: a global perspective. Washington, DC: American Institute for Cancer Research, 1997.
2. Löwik MRH, Hulshof KFAM, Heijden LJM van der, Brussaard JH, Burema J, Kistemaker C, Vries PJF de. Changes in the diet in the Netherlands: 1987-1988 to 1992. Int J Food Sciences 1998: 49(Suppl 1): S1-S64.
3. Krebs-Smith SM, Cook A, Subar AF, Cleveland L, Friday J. US adults' fruit and vegetable intakes, 1989 to 1991: A revised baseline for the *Healthy People 2000* objective. Am J Public Health 1995; 85: 1623-1629.
4. Wearne SJ, Day MJ. Clues for the development of food-based dietary guidelines: how are dietary targets being achieved by UK consumers? Br J Nutrition 1999; 81(Suppl 2): S119-S126.
5. Flynn MA, Kearney JM. An approach to the development of food-based dietary guidelines for Ireland. Br J Nutrition 1999; 81 (Suppl 2): S77-S82.
6. Haraldsdóttir J. Dietary guidelines and patterns of intake in Denmark. Br J Nutrition 1999; 81(Suppl 2): S43-48.
7. Morabia A, Wynder EL. Dietary habits of smokers, people who never smoked, and exsmokers. Am J Clin Nutr 1990; 52: 933-937.
8. Zo eet Nederland 1998 (In Dutch). Den Haag, The Netherlands: Voedingscentrum, 1998.

9. Hulshof KFAM, Staveren WA van. The Dutch National Food Consumption Survey: design, methods and first results. Food Policy 1991; 16: 257-260.
10. Hoogenveen RT, Hollander AEM de, Genugten MLL van. The chronic diseases modelling approach. RIVM report 266750001. Bilthoven, The Netherlands: RIVM, 1998.
11. Genugten M van, Hoogenveen R, Hollander ADE. Chapter 8: Incorporating risk factor epidemiology in mortality projections. In: Forecasting mortality in developed countries. Insights from a statistical, demographic and epidemiological perspective. Tabeau E, Berg Jeths A van den, Heathcote C (Eds.). Dordrecht, The Netherlands: Kluwer Academic Publishers, 2001: 189-204.
12. Klerk M, Jansen MCJF, Veer P van 't, Kok FJ. Fruits and vegetables in chronic disease prevention. Wageningen, The Netherlands: Wageningen Agricultural University, 1998.
13. Centraal Bureau voor de Statistiek (CBS). Maandstatistiek van de bevolking (In Dutch). Voorburg/Heerlen, The Netherlands: CBS, 1994.
14. Centraal Bureau voor de Statistiek (CBS). Vademecum gezondheidsstatiek 1996 (In Dutch). Voorburg/Heerlen, The Netherlands: CBS, 1996.
15. Visser O, Coebergh JWW, Schouten LJ, Dijck JAAM van (Eds.) Incidence of cancer in the Netherlands 1994. Sixth report of the Netherlands Cancer Registry. Vereniging van Integrale Kankercentra, Utrecht, 1997.
16. Valsta LM. Food-based dietary guidelines for Finland – a staged approach. Br J Nutrition 1999; 81(Suppl 2): S49-S55.
17. Becker W. Dietary guidelines and patterns of food and nutrient intake in Sweden. Br J Nutrition 1999; 81(Suppl 2): S113-S117.
18. Hermann-Kunz E, Thamm M. Dietary recommendations and prevailing food and nutrient intakes in Germany. Br J Nutrition 1999; 81(Suppl 2): S61-S69.
19. Volatier J-L, Verger P. Recent national French food and nutrient intake data. Br J Nutrition 1999; 81(Suppl 2): S57-S59.
20. Turrini A, Leclercq C, D'Amicis A. Patterns of food and nutrient intakes in Italy and their application to the development of food-based dietary guidelines. Br J Nutrition 1999; 81(Suppl 2): S83-S99.
21. Serra-Majem L, Ribas L, Ramon JM. Compliance with dietary guidelines in the Spanish population. Results from the Catalan Nutrition Survey. Br J Nutrition 1999; 81(Suppl 2): S105-S112.
22. Steinmetz KA, Potter JD. Vegetables, fruit, and cancer. I. Epidemiology. Cancer Causes Control 1991: 2; 325-357.
23. Block G, Patterson B, Subar A. Fruit, vegetables, and cancer prevention: a review of the epidemiological evidence. Nutr Cancer 1992; 18: 1-29.
24. Trichopoulos D, Willett WC (Eds.). Cancer Causes Control 1996; 7: 3-180.
25. Committee on Medical Aspects of Food and Nutrition, Nutritional aspects of the development of cancer. Policy Report of the Working Group on Diet and Cancer of the Committee on Medical Aspects of Food and Nutrition Policy (COMA-report). Report on Health and Social Subjects, no. 48. London: the Stationery Office, 1998.
26. Doll R, Peto R. The causes of cancer: quantitative estimates of avoidable risks of cancer in the United States today. J Natl Cancer Inst 1981; 66: 1191-1308.
27. Willett WC. Diet, nutrition, and avoidable cancer. Environ Health Perspect 1995; 103(Suppl 8): 165-170.

28. Mooy JM, Gunning-Schepers LJ. Computersimulatie modellen in 'evidence-based' facetbeleid (In Dutch). Amsterdam, The Netherlands: Instituut voor Sociale Geneeskunde, Academisch Medisch Centrum, Universiteit van Amsterdam, 1998.
29. Havas S, Anliker J, Damron D, Langenberg P, Ballesteros M, Feldman R. Final results of the Maryland WIC 5-A-day promotion program. Am J Public Health 1998; 88: 1161-1167.
30. Buller DB, Morrill C, Taren D, Aickin M, Sennott-Miller L, Klein Buller M, Larkey L, Alatorre C, Wentzel TM. Randomized trial testing the effect of peer education at increasing fruit and vegetable intake. J Natl Cancer Inst 1999; 91: 1491-1500.
31. Lutz SF, Ammerman AS, Atwood JR, Campbell MK, DeVellis RF, Rosamond WD. Innovative newsletter interventions improve fruit and vegetable consumption in healthy adults. J Am Diet Assoc 1999; 99: 705-709.
32. Cox DN, Anderson AS, Reynolds J, McKellar S, Lean MEJ, Mela DJ. Take five, a nutrition education intervention to increase fruit and vegetable intakes: impact on consumer choice and nutrient intakes. Br J Nutrition 1998; 80: 123-131.
33. Hoogenveen RT, Jansen MCJF, Houterman S, Verschuren WMM. Changes in consumption of vegetables and fruits, saturated fatty acids and trans fatty acids in The Netherlands: an estimation of the public health effects using the Chronic Diseases Model (In Dutch: Veranderingen in de consumptie van groente en fruit, verzadigd vet en transvetzuren in Nederland: een schatting van de volksgezondheidseffecten met behulp van het Chronische Ziekten Model). RIVM-report number 261858005. Bilthoven, The Netherlands: RIVM, 2001.

Appendix

Relative risks (RR) for fruits and vegetables and cancer in the highest consumption category compared to no risk reduction in the lowest consumption category, i.e., RR=1

Cancer	Best guess RR with consumption of 400 g/d or more	Conservative RR with consumption of 400 g/d or more
Oral cavity	0.45	0.50
Larynx	0.52	0.57
Esophagus	0.54	0.61
Lung	0.58	0.65
Stomach	0.49	0.59
Colon	0.63	0.74
Rectum	0.63	0.74
Bladder	0.65	0.74
Kidney	0.80	0.85
Pancreas	0.62	0.75
Breast	0.84	0.96
Endometrium	0.78	1
Cervix	0.85	1
Ovary	0.85	1
Prostate	0.93	1

Chapter 9

General discussion

Introduction

The studies described in this thesis belong to field of nutritional epidemiology, in which dietary factors are studied in relation to the occurrence of disease, in this thesis epithelial cancer. The aim was to investigate whether intakes of fruits and vegetables are associated with the risk of epithelial cancer of the alimentary and respiratory tracts, particularly of lung cancer, in prospective studies.
In this general discussion, main findings are summarized, methodological issues are discussed, related etiological and public health issues are considered, and directions for future research are given.

Main findings

Higher intakes of dietary fiber were associated with lower colorectal cancer mortality rates among 16 male middle-aged cohorts in Europe, Japan and the United States between 1960-1985 (Chapter 2). Intakes of vitamin B6 and α-tocopherol were also inversely associated with risk, but these intakes were strongly correlated with fiber intake. Consumption of fruits and vegetables and subgroups of these foods were not related to colorectal cancer risk. Fiber intake seemed indicative for that of part of plant food consumption relevant for lowering colorectal cancer risk at the population level.
In similar analyses, fruit intake was inversely associated with 25-year stomach cancer risk and refined grain intake was positively related with this risk (Chapter 3). Consumption of vegetables and other plant food groups was not related with stomach cancer risk. Because of the strong negative correlation between intake of fruits and refined grains in this study, it could not be concluded whether high intakes of refined grains increase stomach cancer risk or that such diets reflect a diet low in fruits.

Fruit intake was inversely related to lung cancer mortality among male smokers aged 50-69 years in Finland, Italy and The Netherlands in a prospective cohort study between 1970 and 1995 (Chapter 4). This association was confined to heavy cigarette smokers. Only in the Dutch cohort, fruit intake was statistically significantly related to lung cancer risk. Vegetable consumption was not associated with risk.
In a national setting, vegetable intake in men and women aged 20-59 years was inversely related to subsequent lung cancer incidence (Chapter 5). This relationship was strong for adenocarcinomas, whereas incidence of Kreyberg I tumors was not statistically significantly associated with vegetable intake. After adjustment for potential

confounders, fruit consumption was not related to lung cancer, mainly due to adjustment for smoking.

Recommendations for fruit and vegetable intake were investigated for their relation with subsequent risk of cancer (total, lung and non-lung epithelial cancer) in Dutch elderly men from Zutphen (Chapter 6). Adherence to the recommended amount of fruits and vegetables was associated with lower total cancer risk. For vegetables, this association was not observed, whereas consuming the recommended 200 grams of fruit per day was related with a lower cancer risk. Variety in vegetable intake however was inversely associated with total cancer and with non-lung epithelial cancer, while variety in fruit intake was not.

Plasma carotenoid concentrations were studied cross-sectionally for their value in categorizing usual vegetable and fruit intakes of Dutch men and women aged 20-59 (Chapter 7). Plasma β-cryptoxanthin concentration best indicated fruit intake, and plasma lutein best divided low and high vegetable consumers. However, carotenoid concentrations were only crude markers of intake and could not distinguish all quartiles of intake as assessed by the food frequency questionnaire used.

In Chapter 8 fruit and vegetable consumption was described for the Dutch population 19 years and older based on the three National Food Consumption Surveys held in 1987/88, 1992 and 1997/98. In 1997/98, the vegetable intake (g/d; mean ± SD) was 142 ± 102 in men and 138 ± 96 in women. For fruits, these figures were 105 ± 122 and 122 ± 121, respectively. Mean levels were below recommended intakes. In the 10-year period between the first and third survey, the mean fruit and vegetable consumption (excluding juices) decreased with 34 g/d (12%) in men and 23 g/d (8%) in women. Fruit and vegetable consumption was lowest and decreased most in this period in the Dutch aged 19-35 with a low level of education. For this group, the potential reduction in cancer incidence over a 40-year period by increasing fruit and vegetable intake to the recommended level of 400 g/d was estimated with a computer simulation to be 14 to 22% at maximum.

An overview of the characteristics and the main findings of the studies described in this thesis are given in Table 1.

Table 1 Studies described in this thesis and their main findings

Chapter	Design	Population	Follow-up	Exposure	Endpoint	Adjusted effect estimate
2	Ecological	16 male cohorts 40-59 yrs from Europe, US and Japan	1960-1985	Consumption of F&V assessed by dietary record; Intake of nutrients chemically analyzed in food composites	Colorectal cancer mortality rate; n=162	RR (95% CI) for ↑ of 10% of mean: F&V not associated; Fiber: 0.89 (0.80-0.97); Vitamin B6: 0.84 (0.71-0.99); α-Tocopherol: 0.94 (0.89-0.99)
3	Ecological	Idem	Idem	Idem	Stomach cancer mortality rate; n=267	RR (95% CI) for ↑ of 10% of mean: Fruit: 0.96 (0.91-0.99); Refined grains: 1.07 (1.03-1.12)
4	Prospective cohort	1,578 Finnish, Italian, Dutch male smokers 50-69 yrs	1970-1995	Consumption of fruits and vegetables in cohort-specific tertiles	Lung cancer mortality; n=149	Fruit consumption in heavy smokers: pooled RR (95% CI) T2: 0.47 (0.26-0.84) and T3: 0.40 (0.20-0.78)
5	Prospective cohort	33,796 Dutch men and women aged 20-59 yrs	1987/91 – 1997; on average 8.4 years	Consumption frequency of fruits and vegetables in quartiles	Lung cancer incidence; n=140	Vegetable consumption: RR (95% CI) Q2: 0.90 (0.59-1.37), Q3: 0.63 (0.39-1.03), Q4: 0.56 (0.33-0.95); In Kreyberg II tumors: T2: 0.38 (0.19-0.76), T3: 0.28 (0.12-0.64)
6	Prospective cohort	730 Dutch elderly men	1985-1995	Adherence to the dietary guidelines for fruit and vegetable intake	Cancer (total, lung and non-lung epithelial) incidence; n=138	Lower cancer risk with recommended F&V/fruit: RR (95% CI) 0.56 (0.31-1.00)/0.62 (0.40-0.96); Lower total/non-lung epithelial cancer risk with vegetable variety: RR (95% CI) 0.64 (0.43-0.95)/0.51 (0.27-0.97)
7	Cross-sectional	591 Dutch men and women aged 20-59 yrs	No follow-up; period 1993-1997	n.a.; Association between F&V intake based on FFQ and plasma carotenoids	n.a.	Fruit associated with β-cryptoxanthin; lutein with vegetable intake; no distinction between different quartiles of intake
8	Computer simulation	Lowly-educated Dutch 19-35 yrs	Simulation over 40 yrs	n.a.; Potential reduction in cancer incidence by F&V	Cancer incidence	Reduction of 14-22% of cancer incidence if everyone would consume F&V according to guidelines

F&V = fruits and vegetables; yrs = years; RR = relative risk; CI = confidence interval; T2-T3 = tertile 2 (intermediate consumption) and 3 (highest consumption); Q2-Q4 = quartile 2 to 4 (4 = highest consumption category); FFQ = food frequency questionnaire; n.a. = not applicable

Methodological considerations

Study designs and potential bias

In observational epidemiology, the main study designs are ecological, case-control and cohort studies. Although every design has its own strengths and weaknesses, the power to evaluate etiological associations increases with study design in the order as given. However, because of the observational nature results should be interpreted in the light of the total amount of evidence available including results from animal and mechanistic experiments.

In ecological analysis, populations are the unit of observation. Results of such studies are of limited value in assessing diet-cancer relationships at the individual level. A relationship observed at the population level does not necessarily imply a similar relationship at the individual level; this is known as "ecological fallacy"[1]. Possibilities to adjust for potential confounders are often limited in ecological studies. An important advantage of this study design is the large potential variation in exposure and outcome.
In Chapter 2 and 3 we performed ecological analyses. Our studies had advantages compared to ecological studies mostly conducted, such as correlation studies using per-capita-disappearance data as proxy for dietary intake and national cancer mortality statistics. We assessed dietary intake in sub-samples of the study population and chemically analyzed the nutrients studied. We investigated the same population for intake and cancer mortality, and used a prospective design. Moreover, we were able to adjust for a few potential confounders. However, our study had some disadvantages: a small number of observations; a long period between dietary assessment and the buying of the foods to be analyzed; one single measurement of food consumption, although characteristic differences between the cohorts seemed still present after 20 years[2].

In case-control and cohort studies associations between diet and cancer are investigated in individuals. In these study designs several possibilities exist to control for potential bias both in design and analysis.
In the case-control approach, subjects are selected on the basis of the presence or absence of the disease under study. Controls and cases should represent the same study population, if not, results will be distorted which is called selection bias. This type of bias is of major concern in case-control studies. Another potential threat to the validity of results of case-control studies is information bias, also called recall bias. This bias appears when disease status influences the information gathered about dietary intake or other lifestyle factors. Illustrative for this potential bias is the strong inverse association observed for vegetable intake in the stomach cancer

cases in the first year of follow-up in The Netherlands Cohort Study, but not in the cases diagnosed in later years[3]. Individuals could have changed their habits because of the disease; this is what occurred in the study of Botterweck et al.[3] for vegetable but not for fruit intake. Results may then be a consequence rather than a cause of the disease.
In a cohort study, subjects are selected who are initially free of disease (under study) and are followed over time. Selection bias is not thought to play a major role in cohort studies. However, when loss to follow-up is not equally distributed over intake and disease categories, this may lead to selection bias. Moreover, if participants of a population-based study differ systematically from the non-participants this is also a form of selection bias, i.e., non-response bias. That recall bias can also play a role in cohort studies was illustrated above[3], however, latency analysis, i.e., excluding the cases in the first (few) year(s) of follow-up, is the analytic answer to most of this potential bias.

The vast majority of literature on the inverse relations between fruit and vegetable intake and epithelial cancers is based on case-control studies. Because selection and information bias are thought to be mainly a problem in case-control studies, results from cohort studies are judged superior to those from case-control studies. Therefore, we studied consumption of fruits and vegetables in relation to epithelial cancer risk in prospective studies. In addition to the biases mentioned, also selective reporting of study findings and publication bias may influence the evaluation of etiological hypotheses.

Besides observational studies, etiological hypotheses can also be tested in intervention studies. Ideally, the effect of fruit and vegetable intake on epithelial cancer should be studied in multiple large randomized trials in human populations. However, such studies may be hardly feasible due to the large numbers of individuals required and the long follow-up period necessary. Even so, because other (dietary) factors will change over time, this approach will not automatically lead to clear answers.

Residual confounding of smoking

Smoking is such a dominant factor in lung cancer etiology that adjustment for this risk factor is a major issue when studying relations between fruit and vegetable intake and lung cancer. Smoking also plays a role in the etiology of stomach cancer and colorectal cancer, however to a smaller extent.
Residual confounding occurs if factors associated with both exposure and outcome are not or insufficiently accounted for in statistical analysis. This insufficient control may result from misclassification of the confounding factor by lack of quality or detail

of the data. In particular if the confounding is strong, as is the case for smoking, misclassification of the confounder can yield spurious associations[4,5].
Smoking is a potential confounder in our results because we observed that smokers consumed less fruits in Chapters 4 and 5 and less vegetables in Chapter 5. In Chapter 6 this association was not reported, but additional analyses showed a statistically significantly lower consumption of fruits and a nonsignificantly lower consumption of vegetables in smokers (data not shown). Many other investigators observed smokers consuming less fruits and vegetables[9-13]. In addition, heavy smokers tend to eat less fruits and vegetables than light smokers[9,11]. These differences seem more pronounced for fruits than vegetables.

Adjusting for the confounding effect of smoking in statistical analyses is mostly done by adjusting for smoking status, intensity, duration, pack-years of smoking or by combinations of these characteristics. Adjustment for smoking status only is considered insufficient. Particularly the number of cigarettes smoked is of importance, but age at starting smoking, type of tobacco or cigarettes smoked and inhalation behavior may influence risk estimates too. Moreover, most smoking data refer to cigarette smoking, although smoking of cigars and pipe is also related to lung cancer risk[6,7]. Boshuizen et al.[8] showed that some residual confounding may remain when adjusting for pack-years only. She found that a model including former smoker, current smoker, number of cigarettes currently smoked, duration of smoking, inhaling and the interaction term 'sex x number of cigarettes currently smoked' adjusted best for smoking in lung cancer analyses in the EPIC-study. Another approach is to stratify analyses on smoking characteristics.
We tried to limit confounding by smoking in several ways. In Chapter 4 analyses were restricted to baseline smokers and adjusted for number of cigarettes smoked, and in addition stratified on smoking intensity. In Chapter 5 and 6 we adjusted for smoking status and pack-years of smoking. In Chapter 5, this adjustment had major impact on the risk estimate for fruit intake. Due to lacking data we could however not adjust for all relevant smoking variables in the different studies. Our results may have also been biased by differential changes in smoking habits during follow-up. The observation from another study that in Finnish cohorts the lighter smokers in 1959 quit smoking during follow-up more often than heavier smokers[14] may illustrate this. Therefore, we cannot rule out residual confounding by smoking in our data, although we adjusted our results for smoking the best we could.

Assessment of fruit and vegetable intake

Several methods can be used for dietary assessment, all with their own advantages and disadvantages[15]. However, regardless of the method, assessing usual fruit and even more so usual vegetable intake in a valid way is very difficult.

We used a food record (Chapter 2, 3 and 8), a cross-check dietary history (Chapter 4 and 6), and food frequency questionnaires (FFQ) (Chapter 5, 6 and 7). The dietary history and the FFQ assess usual consumption. However, the validity of such measurement depends on the participant's memory and ability to take into account the variability in intake, for example from day to day, or by season. Moreover, memory might also be biased by the actual health status. In contrast, food records assess actual intake. In using them, we assumed that the actual consumption was a good indication for the usual intake at the group level.
As described in Chapters 4-7, the reproducibility of the methods used was reasonable but the validity was poor (for vegetables) to moderate (for fruits). Our data on validity were in line with those found by others[16,17]. Thus it may be concluded that fruits and especially vegetables assessed in observational epidemiological studies must be seen as only crude measures of intake, leading to the observation of attenuated associations. Using repeated measurements of intake may improve the measurement of usual intake because random variation is minimized and changes in diet are taken into account. However, more research is necessary to show whether repeated measurements also lead to much better measures of vegetable intake. Perhaps, the widespread choice of FFQs should be reconsidered for measuring vegetable intake. Especially when the aim is not only to compare persons high and low in consumption, but also to study dose-response and adherence to dietary guidelines for the relation with epithelial cancer risk. For frequently used fruits, such as apples, FFQs perform reasonably well, whereas assessment of less frequent or seasonal used fruits, such as strawberries, may be less accurrate.

Because of the difficulty to assess usual fruit and predominantly vegetable intake by dietary assessment methods, biomarkers of dietary intake would be useful tools in studying the relation between these foods and epithelial cancer risk. A main advantage of biomarkers is that they are objective measures[18]. A disadvantage may be the large intra-individual variances observed.
We, like others, studied plasma carotenoids as biomarkers of vegetable and fruit intake (Chapter 7). From intervention studies it is clear that carotenoid concentrations rise when fruit and vegetable intake is increased. However, we showed that carotenoid levels only crudely indicated usual fruit and vegetable intakes.

Study power

Small variations in and/or missing extreme intakes in study populations may be a limiting factor for observational studies in revealing significant relations between epithelial cancers and fruits, vegetables and their related compounds. The advantage of using the data of the Seven Countries Study was the wide variation in

intakes and inclusion of high intakes too. For instance, dietary fiber intake ranged from 21.0-57.2 g/d (Chapter 2), while the median fiber intake in the first vs. the fifth quintile was 9.8 vs. 24.9 g/d in the Nurses Health Study[19] and 14.2 vs. 32.8 g/d in the Physicians Health Study[20].
A limited number of cancer cases can also restrict the power to study the relation between fruit and vegetable consumption and epithelial cancer. This may be a problem for prospective cohort studies, because large numbers of individuals need to be included to result in a substantial number of cancer cases after follow-up. This problem may be especially present when it is intended to study subpopulations or histological types of cancer.

The range in intake and the number of cancer cases, and thus the power can be increased by pooling studies or by extending the geographic area or types of study populations. The way in which such data are appropriately analyzed, i.e., fully using the larger variation while not comparing countries or populations, should be carefully considered. Perhaps, analytical methods should be developed for this purpose. Because by using country or population-specific intake categories the variation in intake in the total study population is not being used.

Etiological considerations

Results of observational studies

Fruits and vegetables and epithelial cancer

In the WCRF-report of 1997 the role of fruits and vegetables in decreasing lung cancer risk was judged 'convincing' but no quantitative risk estimate was given[21]. The conclusion drawn in the COMA-report in 1998 was 'moderately consistent evidence' for fruits and 'weakly consistent evidence' for vegetables with relative risks generally between 0.5-0.7[22]. The prospective cohort studies published since that time[23-28] observed relative risks between 0.58-1.22 for fruits, 0.70-1.0 for types of fruits[25], 0.70-1.04 for vegetables, 0.2-1.1 for types of vegetables[25], and 0.52-1.12 for fruits and vegetables combined in the highest consumption category vs. the lowest. Especially stable high fruit and vegetable intakes were related to lower lung cancer risks[23]. Only the relative risks found for the Physicians' Health Study were above 1. The preliminary results of the EPIC-study presented recently indicated an inverse association for fruits (RR Q4 vs. Q1 0.78, 95% CI 0.58-1.04), but not for vegetables although a protective effect for some vegetable subgroups was suggested[29].

The judgement of the WCRF-panel was a 'convincing' decreased risk for vegetable intake on colorectal cancer and 'inconsistent' for fruits[21]. The COMA-report

judged the association with vegetables as 'moderately consistent' and 'inconsistent' for fruits, and mentioned for vegetable intake a range of relative risks of 0.5-0.9[22]. Furthermore, combined data from the Nurses' Health Study and Health Professionals Follow-up Study did not reveal an association between fruit and vegetable consumption and colon or rectal cancer[30]. In Finland too no association was seen[31]. In the Netherlands Cohort Study, fruit and vegetable intake was inversely associated with colon cancer in women (RR Q5 vs. Q1 0.66, 95% CI 0.44-1.01), but not in men, whereas for rectal cancer no association was seen[32]. In Sweden higher consumption of fruits and vegetables was related with lower colorectal cancer risk (RR Q4 vs. Q1 0.73, 95% CI 0.56-0.96), largely due to fruit (RR Q4 vs. Q1 0.68, 95% CI 0.52-0.89)[33]. Preliminary findings on colorectal cancer in EPIC indicated an inverse association with vegetable intake in men (RR Q5 vs. Q1 0.72; 0.54 after excluding the first two years of follow-up), nonsignificantly inverse in women (RR Q5 vs. Q1 0.77 and 0.67, respectively), and no relation for fruit intake[34].

For stomach cancer, the evidence for a protective effect of both fruits and vegetables was considered 'convincing' in the WCRF-report[21], and 'moderately consistent' in the COMA-report[22]. Recent prospective cohort studies did not yield consistent findings: some showed an inverse association between fruit and vegetable consumption and stomach cancer[35-37], while others did not[3]. Preliminary results of the EPIC-study support a protective effect of fruit intake[38].

Dietary fiber and colon/colorectal cancer

The two review committees judged the role of fiber in decreasing colorectal cancer risk as 'possible'[21] and 'moderately consistent'[22]. In later US prospective studies in men[20] and in women[19] no relationship was found between dietary fiber and risk of colon cancer. In these studies, dietary fiber was also not related to occurrence of colon adenomas in men[39] or women[19]. A lack of association between fiber intake and colon or colorectal cancer has also been seen in other prospective cohort studies[31,33,40]. Preliminary results of EPIC show a strong independent protective effect of fiber on the occurrence of colorectal cancer (RR Q5 vs. Q1 0.6)[41].

Conclusion of results from observational studies

It seems that in recent published cohort studies the associations between fruit, vegetable and fiber intake and epithelial cancers has been weaker than in previous, mostly case-control, studies. Especially the most recent studies conducted in the US provide little evidence for a protective effect of fruit and vegetable consumption. However, the European studies show a more mixed picture, with both inverse and null associations. Possible explanations for this inconsistency are (lack of)

heterogeneity in the study populations or other types of fruits and vegetables consumed.

Results of intervention studies

Large-scale intervention studies of high doses of β-carotene have shown no effect or increased risk of lung cancer[42-44]. An intervention trial in lung cancer patients with vitamin A and *N*-acetylcysteine resulted in no benefit[45]. Intervention with a combination of β-carotene, vitamin E and selenium was followed by lower stomach cancer risk in a high-risk population[46]. Intervention trials among patients with adenomas of the colon and rectum revealed no evidence that fiber intake up to around 30 g/d could prevent the recurrence of adenomas[47,48].

Interventions with fruit and vegetables were conducted only in relatively small intervention trials that studied subsequent plasma concentrations[49,50], markers of DNA damage[51-53] or induction of biotransformation enzymes such as cytochrome P450 and *N*-acetyltransferase 2[54]. Such interventions show that plasma carotenoid and vitamin C levels generally increase, provide some indication that DNA damage may be lowered and revealed mixed results on influencing the activity of biotransformation enzyme activities. Moreover, prediagnostic blood concentrations of carotenoids, such as β-carotene, cryptoxanthin and lutein/zeaxanthin have been found to be lower in subsequent (lung) cancer cases compared to non-cases (for example[55]).

Mechanistic studies are needed to further elucidate the association between fruit and vegetable intake and epithelial cancer, but translating these results to the observational level or vice versa is still difficult.

Fruits and vegetables

The wide variety in fruits and vegetables regarding botanical family, phytochemical content, size, maturity and culinary usage hinders the search for its etiological associations with epithelial cancer. Moreover, this problem is further enhanced by the varied assessment methods used for measuring fruit and vegetable intake.

Fruits and vegetables are studied as total intake, as separate groups, in subgroups, as separate types and as compounds from fruits and vegetables. There is however no universal definition for fruits and vegetables. Especially for potatoes, legumes, nuts and fruit juices practices differ for in- or exclusion. Moreover, no unique classification is used for the definition of subgroups, although a good initiative for such a classification has been taken[56]. In addition, food composition data on hypothesized chemopreventive phytochemicals are mostly not complete and/or valid enough, or even not available at all for assessing intake. In addition, consumption of

fruits and vegetables is mostly lowly correlated. Whether potential cancer preventable actions of fruits and vegetables are interchangeable is not clear.
Epidemiological studies differ in findings for fruits and vegetables or any of the mentioned classifications. In some studies cancer protection was indicated for specific fruit and vegetables groups, such as cruciferous[27] and green leafy vegetables[57], or specific types, like carrots[25] and tomatoes[58], or compounds, such as lutein[59], α-carotene, lycopene and a variety of carotenoids[60]. However, till now, there is not enough evidence to point at a specific part of the fruit and vegetable consumption for cancer prevention for any site.

Variety in intake is in additional aspect of fruit and vegetable consumption. Variety is part of the dietary guidelines for a long time[61], although its value in prevention of epithelial cancer is insufficiently known. It is thought that a combination of compounds, provided by a variety of fruits and vegetables, may be more effective in cancer prevention than single compounds, due to complementary or synergetic mechanisms of action. In Chapter 6 we studied variety in fruit and vegetable intake. We could investigate variety in addition to quantity of intake because correlation between variety and quantity was low due to two different measurements of fruit and vegetable intake. Most other studies looking at variety in fruit and vegetable intake in relation to epithelial cancer risk were case-control studies (see Chapter 6). Moreover, the only cohort study studying variety and lung cancer risk had only one assessment method that could be used, and subsequently experienced a relatively high correlation between consumed amount and variety[28].

Cancer types

As stated in Chapter 1, cancer is a generic name covering many etiologies. Cancers are mostly divided based on topography, like we did in this thesis: cancers of the lung, stomach, and colon and rectum. However, a further classification is possible, for example on histology such as we did in Chapter 5 by dividing Kreyberg I and Kreyberg II tumors. Our findings for the association with vegetable intake differed for these histological types. Also the pattern of (nonsignificant) associations with individual vegetables was totally different for these tumors. Results from other studies by histological type of lung cancer are not consistent, but differences in etiology are indicated. Moreover, the distribution of histological types within lung cancer is not constant, but may change over time[62,63] and may differ between populations (see Chapter 5). Also for stomach cancer, trends in incidences have been observed. In many western countries incidence of adenocarcinomas of the gastric cardia is increasing[64], while the incidence of the intestinal type in the distal part of the stomach has decreased dramatically[65]. Also for these forms etiology is assumed to be different[66].

If associations with fruits and vegetables indeed differ for histological types or locations within the affected organ, differences in study populations related to the distribution and trends in incidences of these types may cloud the etiological picture emerging from studies.

Shape of association

Relationships between fruit and vegetable consumption and epithelial cancers are generally assumed to be linear. However, protective effects may level off at a certain point, or cancer risk may be elevated only at a very low intake of fruits and vegetables; both resulting in a nonlinear relationship. It is possible however that before reaching such a critical point the relationship is indeed linear. In Chapter 4 we tried to study the shape of the relation between fruit and vegetable intake and lung cancer mortality. Country-specific lung cancer mortality rates in smokers were plotted against the medians of the tertiles of fruit intake. From this figure, a log-linear relationship among smokers was indicated. Moreover, Feskanich et al.[28] showed that lung cancer risk did not decrease in women with fruit and vegetable intakes above 5 servings a day and that risk was increased only in those consuming two or fewer servings/day compared to 5 servings/day. Also Terry et al.[33] showed the strongest prevention of colorectal cancer and a more evident dose-response effect in those at the lowest amounts of fruits and vegetables.

Differences in time

Weak and inconsistent associations between fruit and vegetable intake and epithelial cancers may be due to measuring consumption not in the etiologically relevant time frame or to a difference in reference periods in dietary assessment. Fruits and vegetables may exert their action somewhere in the multi-stage cancer process, however, it is not known when in this process their presence is critical. We do know that in carcinogenesis several decades may elapse between start of the disease and diagnosis. However, it is not clear what the correct period, both referring to the baseline measurement as the years of follow-up, for studying relations between intake and cancer should be.

Another dimension of time is the age of the population under study. The incidence of epithelial cancer rises exponentially with age. However, whether aging processes per se play a role in carcinogenesis is still under debate[67,68]. In this thesis, ages from the populations studied varied. In Chapter 5 we investigated relatively young men and women and observed a relatively high incidence of adenocarcinomas of the lung and less squamous cell tumors, an observation consistent with other studies[69]. Besides biological age of the study population which may be related to changes in molecular and physiological processes involved in carcinogenesis[68] and leading to

an accumulation of genetic damage, also lifestyle factors such as fruit and vegetable intake and smoking habits may depend on age. For example, young adults generally eat less fruits and vegetables than older people, but also the choice of fruits and vegetables consumed may differ between age groups. Prevalences of smoking vary with age, and also the type of cigarettes smoked may differ. As an example, it is hypothesized that the rise in adenocarcinomas of the lung may be due to the increased use of low-tar filter cigarettes and the subsequent differences in inhalation behavior[62]. Moreover, cancer is thought of as a multi-factorial disease, therefore changes in other risk factors may lead to changes in associations between fruit and vegetable intake and epithelial cancer.

Geographical differences

Results of epidemiological studies may also differ due to studying the relation between diet and cancer in different parts of the world. This may lead to differences in exposure, for example in varieties of fruits and vegetables consumed, i.e., a relative low intake of cruciferous vegetables in the US compared to The Netherlands; in outcome measurements, for example by differences in diagnosis, registries and care or differences in the distribution of histological types; in other risk factors an thus potential confounders, such as other dietary and lifestyle factors, but also prevalences of viruses and less concrete constructs as prosperity, for example illustrated by the geographical trend in introduction of refrigerators followed by decreasing trends in stomach cancer which started in the US, followed by Europe and later by Asia.

All these factors may influence the association between fruit and vegetable intake and epithelial cancer. On the other hand, just because the inverse association between fruit and vegetable intake and epithelial cancer is observed in populations around the world with widely differing lifestyles, this also provides strong evidence in favor of this hypothesis.

Other differences in study populations

As mentioned above, by different distributions of other risk factors in study populations, observed associations may vary. It is tried to eliminate such effects by adjusting for these factors in statistical analyses. However, because most risk estimates are relative risks, the measure always directly depend on the 'baseline risk' in the reference group. In Chapter 4 we calculated both relative and absolute risks. The absolute risks indicated that among smokers, fruit intake may be only inversely associated with lung cancer mortality in those with low intake and at high absolute risk. Another example is that higher folate intake from fruits and vegetables is strongest inversely related to colon cancer risk among persons who regularly consume alcohol, which itself is associated with risk of this cancer[70]. Perhaps such findings

indicate that only higher absolute risks may be lowered by fruit and vegetable consumption.

Public health considerations

Potential cancer prevention

Some but not all associations we studied between fruit and vegetable consumption and epithelial cancer risk were statistically significantly inverse. These findings resemble the picture generally found in literature: most risk estimates are in the protective direction, but not all are statistically significant. Given the difficulty of assessing fruit and vegetable intake and all the differences between studied populations, it can be concluded that results from observational epidemiological studies still indicate an inverse association between fruit and vegetable intake and epithelial cancer. However, these relations seem weaker than halving the risk as thought in the beginning of the nineties[71]. An important explanation for this attenuation may be that the vast majority of the older literature was based on case-control studies. However, also residual confounding by smoking, inadequate adjustment for other potential confounders, the choice of study populations, trends over time of factors other than fruit and vegetable intake, variety in fruit and vegetable intake, and publication bias may have influenced the risk estimates.
Estimating up-to-date relative risks for the relations between fruit and vegetable consumption and epithelial cancer is risky. However, based on the available literature relative risks of approximately 0.75 for lung cancer, 0.80 for stomach cancer and 0.85 for colorectal cancer for the highest consumption category compared to the lowest category could be estimated. We presume that the highest consumption category corresponds with the dietary guideline of consuming 400 g/d of fruits and vegetables and the lowest category with less than 100 g/d. The evidence seems somewhat stronger for fruit intake in lung and stomach cancer, while colorectal cancer seems more associated with vegetable consumption. However, because of the inconsistency in results we do not estimate relative risks for fruits and vegetables separately.

To which cancer reduction at the population level these relative risks may lead depends amongst others on the consumption level of fruits and vegetables and the occurrence of these cancer types. For a risk group within the Dutch population we estimated a maximum reduction of 14-22% in cancer incidence over 40 years when all would adhere to the guideline for fruit an vegetable intake (Chapter 8). This calculation included also the potential reduction of cancers other than lung, stomach, and colon and rectum. Moreover, because only 72% of the incident cancers were

included in this computer simulation, the percentage reduction of total cancer is expected to be lower. Because the studied population had relatively low intakes of fruits and vegetables, the theoretically expected cancer reduction in the general population may be even less. In addition, the practical achievable reduction may be even lower than that, as illustrated by the findings of the other scenarios we simulated in Chapter 8.

Riboli and Norat estimated that an increase of the average population consumption of fruit and vegetables up to the level corresponding to the 75th percentile of current consumption, may prevent 10 to 50% of the digestive tract cancers[72]. Observed population-level changes in five risk factors for colon cancer, i.e., vegetable intake, red meat intake, alcohol consumption, physical activity levels, and weight status were modeled for the US adult population over the years 1975-1995 to evaluate their impact on population risk of colon cancer[73]. Increased vegetable intake was expected to have reduced colon cancer incidence with 1.3-4.0% (depending of assumptions) in 1995 relative to 1985. The theoretical optimum reduction, which would be obtained if the entire population moved to the lowest risk category, was expected to be 32.7%. When trends in all five factors were considered together, the estimation for the overall reduction was around zero, showing very little change from 1985 to 1995. This finding was however not consistent with the recently observed decline in colon cancer in the US.

Dietary recommendations

Dietary recommendations have been originally formulated to meet nutrient requirements and to prevent deficiency diseases. Nowadays prevention from chronic diseases like different types of cancer is used as an additional rationale to promote fruit and vegetable consumption. The Dutch recommendation has been set at 200 grams of fruits and 150-200 grams of vegetables daily for those 12 years and older. Many countries recommend 400 grams of fruits and vegetables per day. Some western countries have set this recommendation somewhat higher, for example Denmark (600 g), Finland (450 g), Belgium (at least 200 g of fruits and 300 g of vegetables), while others have not formulated dietary guidelines for these foods yet, like Austria[74]. For estimating the proportion of the population adhering to the dietary guidelines, many data on fruit and vegetable intake are not appropriate[75].

Up till now, there is no evidence that fruit and vegetable intakes much higher than 400 g/d will result in even further lowering of epithelial cancer risk compared to the recommended level. However, there is also no evidence that such higher intakes have adverse effects.

Besides potential prevention of cancer, there are more reasons to recommend (higher) intakes of fruits and vegetables. The risk of other chronic diseases such as

coronary heart disease[76,77], chronic obstructive pulmonary disease[78] and cataract[79] may also be inversely related to fruit and vegetable intake.

Campaigns to promote consumption

In 1991 the "5 A Day for Better Health" Program was started in the US to promote fruit and vegetable consumption[80]. Since the implementation of this program Americans increased consumption of fruits and vegetables but dark green and cruciferous vegetable intake is still low[81]. Other countries followed the US initiative of a promotion campaign, among others The Netherlands in 1995.
It seems however difficult to increase fruit and vegetable consumption in the long run. Programs mostly achieve a rise of around half a serving per day, i.e., around 40 grams[82,83]; sometimes somewhat higher up to around one serving[84,85]. In a Dutch study it was found that increasing self-efficacy and positive attitudes towards fruit and vegetable consumption may result in higher intakes[86]. In the US, number of servings individuals think they should have during a day, liking the taste, and having the habit of eating lots of fruits and vegetables since childhood were indicated as important factors related to fruit and vegetable intakes[87]. A major obstacle to nutrition education is that the majority of the people believes that they already eat healthy[88]. Attitudes about a healthy diet are assumed to be more important than knowledge[12].

Future research directions

One of the possibilities to overcome some of the methodological considerations mentioned is to increase the power of the study, by increasing the size of the study and the variation in intake levels. Many large prospective studies are currently underway[70]. The study power can also be enhanced by pooling studies or results of studies. For the relation between nutrition and cancer this is done in The Pooling Project coordinated by Harvard[89]. Another way is to coordinate assessment of dietary intake in several countries or populations such as be done in the multi-center prospective cohort study European Prospective Investigation into Cancer and Nutrition (EPIC) coordinated by the International Agency for Research on Cancer (IARC) in Lyon, France[90].

However, when assessment of fruit and vegetable intake or other potential confounders is not valid, an increased study power will not help to further elucidate the role of fruits and vegetables in epithelial cancer etiology. Especially measuring vegetable intake appears extremely difficult. Therefore, full attention must be paid to the development of methods which assess usual intake of fruits, but particularly of vegetables, better then most FFQs generally used in prospective cohort studies.

Probably, a combination of methods, including biomarkers of intake, is the way to go. The search for proper measurement tools should also consider the question at which points in time should intake be assessed.
In addition, to further explore the shape of the relation and to come to a dose-response relationship between fruit and vegetable intake and epithelial cancer, if applicable, an accurate assessment of portion sizes is needed. Unless such information, it is impossible to make statements about whether intakes of 400-800 g/d of fruits and vegetables may further lower cancer risk, as has been done in the WCRF-report[21]. Because it is still not clear whether a possible cancer-lowering effect of fruits and vegetables is caused by specific compounds or by the combination of many different compounds, the role of variety in fruit and vegetable consumption should be included in future research.
In cancer epidemiology, relative risks are generally calculated as effect measures. However, reference groups differ in every study, which interferes with the comparability between studies. Using absolute risk estimates would enhance this comparability, and therefore help in getting more insight in the relation between fruit and vegetable intake and epithelial cancer.

Because of the long latency period of cancer, intermediate markers of cancer would be extremely helpful in studying the relation between fruit and vegetable intake and subsequent epithelial cancer. Adenomas of the colon are widely used as intermediate marker for colon cancer. However, it is known that not all adenomas develop into malignant tumors, and it is thought that associations with diet differ in development of adenomas compared to development of tumors. In studying stomach cancer, precursor stages such as metaplasia and dysplasia are used. Also markers of earlier stages in the carcinogenesis are used in epidemiological studies, like markers of DNA damage such as DNA adducts in the lung. More knowledge about such intermediate markers and their relevance to the final development of epithelial cancer is needed.

Research efforts and knowledge into the field of genetics have grown explosively in recent years. In cancer research, the interplay between genes such as oncogenes, tumor suppressor genes, DNA-repair genes and genes involved in activation and detoxification with vegetable and fruit consumption is investigated. Some studies indicate stronger associations between epithelial cancer and fruit and vegetable intake for certain genotypes. Although this research will certainly lead to more mechanistic understanding, its role in improving public health is questionable for the time being. The road to greater knowledge about measures for prevention and therapy is still long, and the acceptance of gene therapy by the public is uncertain.

Most important discoveries of the past two decades in cancer epidemiology relate to the carcinogenic effects of infectious pathogens[67]. *Helicobacter pylori* is now seen as a major risk factor for stomach cancer. In addition, incidence of epithelial cancers of the lung, colon and rectum is increased in immunosuppressed patients, suggesting that unidentified viruses may also play a role in these cancers[67]. Some cancers may therefore be prevented or postponed by vaccination with tumor-specific antigens or by less specific immunostimulation[91,92]. The role of enhancing immune function in lowering epithelial cancer risk is promising and certainly needs further investigation.

Stopping or better not starting smoking is the first way to lower epithelial cancer risk. In addition, eating at least 400 grams of a wide variety of fruits and vegetables daily, maintaining a normal weight, and not drinking alcohol excessively will help to further lower epithelial cancer risk. However, it is not realistic to expect that every person in a population will reach this optimal picture. Perhaps, the potential role of increasing immune function in lowering the risk of epithelial cancer may become an additional aid.

References

1. Morgenstern H. Uses of ecologic analysis in epidemiologic research. Am J Publ Health 1982; 72: 1336-1344.
2. Kromhout D, Keys A, Aravanis C, Buzina R, Fidanza F, Giampaoli S, Jansen A, Menotti A, Nedeljkovic S, Pekkarinen M, Simic BA, Toshima H. Food consumption patterns in the 1960s in seven countries. Am J Clin Nutr 1989; 49: 889-894.
3. Botterweck AAM, Brandt P van den, Goldbohm RA. A prospective cohort study on vegetable and fruit consumption and stomach cancer risk in the Netherlands. Am J Epidemiol 1998; 148: 842-853.
4. Rothman KJ, Greenland S. Precision and validity in epidemiologic studies. In: Rothman KJ, Greenland S (Eds.) Modern Epidemiology. Philadelphia, US: Lippincott-Raven, 1998: 115-134.
5. Marshall JR, Hastrup JL. Mismeasurement and the resonance of strong confounders: uncorrelated errors. Am J Epidemiol 1996; 143: 1069-1078.
6. Boffetta P, Pershagen G, Jöckel K-H, Forastiere F, Gaborieau V, Heinrich J et al. Cigar and pipe smoking and lung cancer risk: a multicenter study from Europe. J Natl Cancer Inst 1999; 91: 697-701.
7. Shapiro JA, Jacobs EJ, Thun MJ. Cigar smoking in men and risk of death from tobacco-related cancers. J Natl Cancer Inst 2000; 92: 333-337.
8. Boshuizen HC, Bueno-de-Mesquita HB, Altenburg HP, Agudo A, LeMarchand L, Berrino F et al. Adjustment for smoking in lung cancer analyses in the EPIC cohort (Abstract 8.03). European Conference on Nutrition & Cancer, June 21-24 2001, Lyon, France.

9. Morabia A, Wynder EL. Dietary habits of smokers, people who never smoked, and exsmokers. Am J Clin Nutr 1990; 52: 933-937.
10. Subar AF, Harlan LC, Mattson ME. Food and nutrient intake differences between smokers and non-smokers in the US. Am J Public Health 1990; 80: 1323-1329.
11. Margetts BM, Jackson AA. Interactions between people's diet and their smoking habits: the dietary and nutritional survey of British adults. BMJ 1993; 307: 1381-1384.
12. Thompson RL, Margetts BM, Speller VM, McVey D. The Health Education Authority's health and lifestyle survey 1993: who are the low fruit and vegetable consumers? J Epidemiol Community Health 1999; 53: 294-299.
13. Ma J, Hampl JS, Betts NM. Antioxidant intakes and smoking status: data from the Continuing Survey of Food intakes by individuals 1994-1996. Am J Clin Nutr 2000; 71: 774-780.
14. Pekkanen J, Tervahauta M, Nissinen A, Karvonen MJ. Does the predictive value of baseline coronary risk factors change over a 30-year follow-up? Cardiology 1993; 82: 181-190.
15. Willett W. Nutritional Epidemiology. Second edition. New York/Oxford: Oxford University Press, 1998.
16. Ocké MC, Bueno-de-Mesquita HB, Goddijn HE, Jansen AM, Pols MA, Staveren van WA, Kromhout D. The Dutch EPIC food frequency questionnaire. I. Description of the questionnaire, and relative validity and reproducibility for food groups. Int J Epidemiol 1997; 26(Suppl 1): S37-S48.
17. Kaaks R, Slimani N, Riboli E. Pilot phase studies on the accuracy of dietary intake measurements in the EPIC Project: Overall evaluation of results. Int J Epidemiol 1997; 26(Suppl 1): S26-S36.
18. Kaaks R, Riboli E, Sinha R. Biochemical markers of dietary intake. In: Application of biomarkers in cancer epidemiology. Toniolo P, Boffetta P, Shuker DEG, et al. (Eds.) IARC Scientific Publications No. 142. Lyon, France: IARC, 1997: 103-126.
19. Fuchs CS, Giovannucci EL, Colditz GA, Hunter DJ, Stampfer MJ, Rosner B, Speizer FE, Willett WC. Dietary fiber and the risk of colorectal cancer and adenoma in women. N Engl J Med 1999; 340: 169-176.
20. Giovanucci E, Rimm EB, Stampfer MJ, Colditz GA, Ascherio A, Willett WC. Intake of fat, meat, and fiber in relation to risk of colon cancer in men. Cancer Res 1994; 54: 2390-2397.
21. World Cancer Research Fund/American Institute for Cancer Research. Food, nutrition and the prevention of cancer: a global perspective. Washington, US: American Institute for Cancer Research, 1997.
22. Report of the Working Group on Diet and Cancer of the Committee on Medical Aspects of Food and Nutrition Policy (COMA-report). Nutritional aspects of the development of cancer. Report on Health and Social Subjects, no. 48. London: The Stationery Office, 1998.
23. Ocké MC, Bueno-de-Mesquita HB, Feskens EJ, Staveren van WA, Kromhout D. Repeated measurements of vegetables, fruits, and antioxidant (pro)vitamins in relation to lung cancer (The Zutphen Study). Am J Epidemiol 1997; 145: 358-365.
24. Yong L-C, Brown CC, Schatzkin A, Dresser CM, Slesinski MJ, Cox CS, Taylor PR. Intake of vitamins E, C, and A and risk of lung cancer. The NHANES I Epidemiologic Followup Study. Am J Epidemiol 1997; 146: 231-243.

25. Speizer FE, Colditz GA, Hunter DJ, Rosner B, Hennekens C. Prospective study of smoking, antioxidant intake, and lung cancer in middle-aged women (USA). Cancer Causes Control 1999; 10: 475-482.
26. Knekt P, Järvinen R, Teppo L, Aromaa A, Seppänen R. Role of various carotenoids in lung cancer prevention. J Natl Cancer Inst 1999; 91: 182-184.
27. Voorrips LE, Goldbohm RA, Verhoeven DTH, Poppel van GAFC, Sturmans F, Hermus RJJ, Brandt van den PA. Vegetable and fruit consumption and lung cancer risk in the Netherlands Cohort Study on Diet and Cancer. Cancer Causes Control 2000; 11: 101-115.
28. Feskanich D, Ziegler RG, Michaud DS et al. Prospective study of fruit and vegetable consumption and risk of lung cancer among men and women. J Natl Cancer Inst 2000; 92: 1812-1823.
29. Miller AB on behalf of EPIC. Presentation at the European Conference on Nutrition & Cancer, June 21-24 2001, Lyon, France.
30. Michels KB, Giovannucci E, Joshipura KJ, Rosner BA, Stampfer MJ, Fuchs CS et al. Fruit and vegetable consumption and colon cancer risk in men and women. J Natl Cancer Inst 2000; 92: 1740-1752.
31. Pietinen P, Malila N, Virtanen M, Hartman TJ, Tangrea JA, Albanes D, Virtamo J. Diet and risk of colorectal cancer in a cohort of Finnish men. Cancer Causes Control 1999; 10: 387-396.
32. Voorrips LE, Goldbohm RA, Poppel G van, Sturmans F, Hermus RJJ, Brandt PA van den. Vegetable and fruit consumption and risks of colon and rectal cancer in a prospective cohort study. Am J Epidemiol 2000; 152: 1081-1092.
33. Terry P, Giovannucci E, Michels KB, Bergkvist L, Hansen H, Holmberg L, Wolk A. Fruit, vegetables, dietary fiber, and risk of colorectal cancer. J Natl Cancer Inst 2001; 93: 525-533.
34. Bueno-de-Mesquita HB on behalf of EPIC. Presentation at the European Conference on Nutrition & Cancer, June 21-24 2001, Lyon, France.
35. Zheng W, Sellers TA, Doyle TJ, Kushi LH, Potter JD, Folsom AR. Retinol, antioxidant vitamins, and cancers of the upper digestive tract in a prospective cohort study of postmenopausal women. Am J Epidemiol 1995; 142: 955-960.
36. Galanis DJ, Kolonel LN, Lee J, Nomura A. Intakes of selected foods and beverages and the incidence of gastric cancer among the Japanese residents of Hawaii: a prospective study. Int J Epidemiol 1998; 27: 173-180.
37. Terry P, Nyren O, Yuen J. Protective effect of fruits and vegetables on stomach cancer in a cohort of Swedish twins. Int J Cancer 1998; 76: 35-37.
38. Gonzalez C, on behalf of EPIC. Presentation at the European Conference on Nutrition & Cancer, June 21-24 2001, Lyon, France.
39. Platz EA, Giovannucci E, Rimm EB, Rockett HRH, Stampfer MJ, Colditz GA, Willett WC. Dietary fiber and distal colorectal adenoma in men. Cancer Epidemiol Biomarkers Prev 1997; 6: 661-670.
40. Gaard M, Tretli S, Løken EB. Dietary factors and risk of colon cancer: a prospective study of 50,535 young Norwegian men and women. Eur J Cancer Prev 1996; 5: 445-454.

41. Bingham S, on behalf of EPIC. Presentation at the European Conference on Nutrition & Cancer, June 21-24 2001, Lyon, France.
42. The Alpha-tocopherol, β-carotene cancer prevention study group. The effect of vitamin E and β-carotene on the incidence of lung cancer and other cancers in male smokers. N Engl J Med 1994; 330: 1029-1035.
43. Hennekens CH, Buring JE, Manson JE, et al. Lack of effect of long-term supplementation with β-carotene on the incidence of malignant neoplasms and cardiovascular disease. N Engl J Med 1996; 334: 1145-1149.
44. Omenn GS, Goodman GE, Thornquist MD, et al. Effects of a combination of β-carotene and vitamin A on lung cancer and cardiovascular disease. N Engl J Med 1996; 334: 1150-1155.
45. Zandwijk N van, Dalesio O, Patorino U, Vries N de, Tinteren H van. EUROSCAN, a randomized trial of vitamin A and *N*-acetylcysteine in patients with head and neck cancer or lung cancer. J Natl Cancer Inst 2000; 92: 977-986.
46. Blot WJ, Li J-Y, Taylor PR, Guo W, Dawsey S, Wang G-Q et al. Nutrition intervention trials in Linxian, China: supplementation with specific vitamin/mineral combinations, cancer incidence, and disease-specific mortality in the general population. J Natl Cancer Inst 1993; 85: 1483-1492.
47. Alberts DS, Martinez ME, Roe DJ, Guillen-Rodriguez JM, Marshall JR, Leeuwen van JB et al. Lack of effect of a high-fiber cereal supplement on the recurrence of colorectal adenomas. Phoenix Colon Cancer Prevention Physicians' Network. N Engl J Med 2000; 342; 1156-1162.
48. Schatzkin A, Lanza E, Corle D, Lance P, Iber F, Caan B et al. Lack of effect of a low-fat, high-fiber diet on the recurrence of colorectal adenomas. Polyp Prevention Trial Study Group. N Engl J Med 2000; 342: 1149-1155.
49. Zino S, Skeaff M, Williams S, Mann J. Randomised controlled trial of effect of fruit and vegetable consumption on plasma concentrations of lipids and antioxidants. BMJ 1997; 314: 1787-1791.
50. Broekmans WM, Klopping-Ketelaars IA, Schuurman CR, Verhagen H, Berg H van den, Kok FJ, Poppel G van. Fruits and vegetables increase plasma carotenoids and vitamins and decrease homocysteine in humans. J Nutr 2000; 130: 1578-1583.
51. Verhagen H, Poulsen HE, Loft S, Poppel G van, Willems MI, Bladeren PJ van. Reduction of oxidative DNA-damage in humans by brussels sprouts. Carcinogenesis 1995; 16: 969-970.
52. Pool-Zobel BL, Bub A, Muller H, Wollowski I, Rechkemmer G. Consumption of vegetables reduces genetic damage in humans: first results of a human intervention trial with carotenoid-rich foods. Carcinogenesis 1997; 18: 1847-1850.
53. Thompson HJ, Heimendinger J, Haegele A, Sedlacek SM, Gillette C, O'Neill CO et al. Effect of increased vegetable and fruit consumption on markers of oxidative cellular damage. Carcinogenesis 1999; 20: 2261-2266.
54. Lampe JW, King IB, Li S, Grate MT, Barale KV, Chen C, Feng Z, Potter JD. Brassica vegetables increase and apiaceous vegetables decrease cytochrome P450 1A2 activity in humans: changes in caffeine metabolite ratios in response to controlled vegetable diets. Carcinogenesis 2000; 21: 1157-1162.

55. Comstock GW, Alberg AJ, Huang H-Y, Wu K, Burke AE, Hoffman SC et al. The risk of developing lung cancer associated with antioxidants in the blood: ascorbic acid, carotenoids, α-tocopherol, selenium, and total peroxyl radical absorbin capacity. Cancer Epidemiol Biomarkers Prev 1997; 6: 901-916.
56. Smith SA, Campbell DR, Elmer PJ, Martini MC, Slavini JL, Potter JD. The University of Minnesota Cancer Prevention Research Unit vegetable and fruit classification scheme (United States). Cancer Causes Control 1995; 6: 292-302.
57. Steinmetz KA, Potter JD, Folsom AR. Vegetables, fruit, and lung cancer in the Iowa Women's Health Study. Cancer Res 1993; 53: 536-543.
58. Franceschi S, Bidoli E, LaVecchia C, Talamini R, D'Avanzo B, Negri E. Tomatoes and risk of digestive-tract cancers. Int J Cancer 1994; 59: 181-184.
59. Slattery ML, Benson J, Curtin K, Ma K-N, Schaeffer D, Potter JD. Carotenoids and colon cancer. Am J Clin Nutr 2000; 71: 575-582.
60. Michaud DS, Feskanich D, Rimm EB, Colditz GA, Speizer FE, Willett WC, Giovannucci E. Intake of specific carotenoids and lung cancer in 2 prospective US cohorts. Am J Clin Nutr 2000; 72: 990-997.
61. Haughton B, Gussow JD, Dodds JM. An historical study of the underlying assumptions for United States food guides from 1917 through the basic four food group guide. J Nutr Educ 1987; 19: 169-76.
62. Janssen-Heijnen MLG, Coebergh JWW, Klinkhamer PJJM, Schipper RM, Splinter TAW, Mooi WJ. Is there a common etiology for the rising incidence of and decreasing survival with adenocarcinoma of the lung? Epidemiology 2001; 12: 256-258.
63. Devesa SS, Shaw CL, Blot WJ. Changing patterns of lung cancer incidence by histological type. Cancer Epidemiol Biomarkers Prev 1991; 1: 29-34.
64. Blot WJ, Devesa SS, Kneller RW, Fraumeni JF, Jr. Rising incidence of adenocarcinoma of the esophagus and gastric cardia. JAMA 1991; 265: 1287-1289.
65. Howson CP, Hiyama T, Wynder EL. The decline in gastric cancer: epidemiology of an unplanned triumph. Epidemiol Rev 1986; 8: 1-27.
66. Botterweck AAM. Diet and risk of stomach cancer (Thesis), 2000.
67. Peto J. Cancer epidemiology in the last century and the next decade. Nature 2001; 411; 390-395.
68. DePinho RA. The age of cancer. Nature 2000; 408: 248-254.
69. Kreuzer M, Kreienbrock L, Gerken M, Heinrich J, Bruske-Hohlfeld I, Muller K-M, Wichmann HE. Risk factors for lung cancer in young adults. Am J Epidemiol 1998; 147: 1028-1037.
70. Willett WC. Diet and cancer: One view at the start of the millennium. Cancer Epidemiol Biomarkers Prev 2001; 10: 3-8.
71. Block G, Patterson B, Subar A. Fruit, vegetables, and cancer prevention: A review of the epidemiological evidence. Nutr Cancer 1992; 18: 1-29.
72. Riboli E, Norat T. Estimation of the proportion of cancers preventable by dietary changes (Abstract 0.33). European Conference on Nutrition & Cancer, June 21-24 2001, Lyon, France.
73. Cronin KA, Krebs-Smith SM, Feuer EJ, Troiano RP, Ballard-Barbash R. Evaluating the impact of population changes in diet, physical activity, and weight status on population risk for colon cancer (United States). Cancer Causes Control 2001; 12: 305-316.

74. Br J Nutrition 1999; 81(Suppl 2).
75. Field AE, Colditz GA, Kay Fox M, Byers T, Serdula M, Bosch RJ, Peterson KE. Comparison of 4 questionnaires for assessment of fruit and vegetable intake. Am J Public Health 1998; 88: 1216-1218.
76. Ness AR, Powles JW. Fruit and vegetables, and cardiovascular disease: a review. Int J Epidemiol 1997; 26: 1-13.
77. Liu S, Manson JE, Lee I-M, Cole SR, Hennekens CH, Willett WC, Buring JE. Fruit and vegetable intake and risk of cardiovascular disease: the Women's Health Study. Am J Clin Nutr 2000; 72: 922-928.
78. Smit HA, Grievink L, Tabak C. Dietary influences on chronic obstructive lung disease and asthma: a review of the epidemiologic evidence. Proc Nutr Soc 1999; 58: 309-319.
79. Tavani A, Negri E, LaVecchia C. Food and nutrient intakes and risk of cataract. Ann Epidemiol 1996; 6: 41-46.
80. Heimendinger J, Van Duyn MA, Chapelsky D, Foerster S, Stables G. The national 5 A Day for Better Health Program: a large-scale nutrition intervention. J Public Health Manag Pract 1996;2:27-35.
81. Johnston CS, Taylor CA, Hampl JS. More Americans are eating "5 a Day" but intakes of dark green and cruciferous vegetables remain low. J Nutr 2000; 130: 3063-3067.
82. Havas S, Anliker J, Damron D, Langenberg P, Ballesteros M, Feldman R. Final results of the Maryland WIC 5-A-day promotion program. Am J Public Health 1998; 88: 1161-1167.
83. Buller DB, Morrill C, Taren D, Aickin M, Sennott-Miller L, Klein Buller M, Larkey L, Alatorre C, Wentzel TM. Randomized trial testing the effect of peer education at increasing fruit and vegetable intake. J Natl Cancer Inst 1999; 91: 1491-1500.
84. Lutz SF, Ammerman AS, Atwood JR, Campbell MK, DeVellis RF, Rosamond WD. Innovative newsletter interventions improve fruit and vegetable consumption in healthy adults. J Am Diet Assoc 1999; 99: 705-709.
85. Cox DN, Anderson AS, Reynolds J, McKellar S, Lean MEJ, Mela DJ. Take five, a nutrition education intervention to increase fruit and vegetable intakes: impact on consumer choice and nutrient intakes. Br J Nutrition 1998; 80: 123-131.
86. Brug J, Lechner L, Vries H de. Psychosocial determinants of fruit and vegetable consumption. Appetite 1995; 25: 285-296.
87. Krebs-Smith SM, Heimendinger J, Patterson BH, Subar AF, Kessler R, Pivonka E. Psychosocial factors associated with fruit and vegetable consumption. Am J Health Promot 1995; 10: 98-104.
88. Kearney JM, McElhone S. Perceived barriers in trying to eat healthier – results of a pan-EU consumer attitudinal survey. Br J Nutrition 1999; 81(Suppl 2): S133-S137.
89. Hunter DJ, Spiegelman D, Adami H-O, et al. Cohort studies of fat intake and the risk of breast cancer: a pooled analysis. N Engl J Med 1996; 334: 356-361.
90. Riboli E, Kaaks R. The EPIC Project: rationale and study design. European Prospective Investigation into Cancer and Nutrition. Int J Epidemiol 1997; 26(Suppl 1): S6-S14.
91. Rosenberg SA. Progress in human tumour: immunology and immunotherapy. Nature 2001; 411: 380-384.
92. Appelbaum FR. Haematopoietic cell transplantation as immunotherapy. Nature 2001; 411: 385-389.

Summary

Epidemiological studies indicated an inverse association between fruit and vegetable intake and epithelial cancers. However, most of these studies used a case-control design, which is prone to selection and information bias. Therefore, the question was raised whether these observations could also be found in prospective studies.

In this thesis, prospective studies on intake of fruits, vegetables and to a lesser extent other plant foods and subsequent epithelial cancer risk were described. The main research question was whether higher intakes of fruits and vegetables were related to lower risks of epithelial cancers, mainly of lung cancer. Moreover, it was investigated whether such inverse associations could be attributed to subgroups or components of plant foods. Also the variety in consumption was studied for its relation with cancer risk. Furthermore, the value of plasma carotenoid concentrations for categorizing subjects on usual vegetable and fruit consumption was investigated. Finally, fruit and vegetable consumption in the Dutch adult population was described, and the potential reduction in cancer incidence by increasing fruit and vegetable consumption was estimated for those 19-35 year of age with a low level of education.

Intake of plant foods in the broad sense, i.e., besides fruits and vegetables also grains and potatoes, were studied in relation to colorectal cancer mortality in 12,763 middle-aged men in the 16 cohorts of the Seven Countries Study (Chapter 2). In this ecological study, risk ratios (RR) for 25-year colorectal cancer mortality were calculated for a change of 10% of the mean intake of plant foods and components. Information about food intake was derived from sub-samples within cohorts using the record method around 1960, whereas dietary components were chemically analyzed in food composites of the average diet per cohort. Consumption of total plant foods, fruits and vegetables were not related to colorectal cancer risk. Fiber intake was inversely associated with colorectal cancer mortality (RR 0.89, 95% confidence interval (CI) 0.80-0.97); corresponding with a 33% lower risk for a 10 g/d higher fiber intake. Intakes of vitamin B6 (RR 0.84, 95% CI 0.71-0.99) and α-tocopherol (RR 0.94, 95% CI 0.89-0.99) were also inversely associated with risk. However, these intakes were highly correlated with fiber intake. Fiber intake seemed to indicate that part of plant food consumption relevant for lowering colorectal cancer risk at the population level.

A similar ecological analysis was performed for the relation between intake of plant foods and stomach cancer in Chapter 3. Again in the Seven Countries Study, risk ratios were calculated for 10% of the mean intakes of total plant food and subgroups, among these fruits and vegetables. Fruit intake was inversely associated with stomach cancer risk (RR 0.96, 95% CI 0.91-0.99) and refined grain intake was positively related with risk (RR 1.07, 95% CI 1.03-1.12). However, low intake of fruits was strongly correlated with high refined grain consumption. The other plant food groups showed no relation with 25-year stomach cancer mortality. Although high intake of refined grains may increase stomach cancer risk, such a diet may just reflect the effect of a diet low in fruits.

Fruit and vegetable consumption was studied in relation to 25-year lung cancer mortality in a cohort of European men in Chapter 4. Around 1970, dietary intake of Finnish, Italian and Dutch men aged 50-69 was assessed. Baseline information was complete for 3,108 men, among which were 1,578 smokers. Fruit consumption was inversely associated with lung cancer mortality among smokers; adjusted relative risks (RR) were 0.56 (95% CI 0.37-0.84) and 0.69 (95% CI 0.46-1.02), for the intermediate and highest tertiles of the individual cohorts, respectively. Only in the Dutch cohort, this association was statistically significant: RRs 0.33 (95% CI 0.16-0.70) and 0.35 (95% CI 0.16-0.74), respectively. Stratifying on cigarette smoking intensity (non, light and heavy) revealed an inverse association in heavy smokers only. Vegetable consumption was not related to lung cancer risk in smokers. In this prospective analysis among European smoking men, fruit intake was inversely related to lung cancer mortality. This association was confined to heavy cigarette smokers.

Dutch men and women aged 20-59 were studied for the relation fruit and vegetable intake and lung cancer incidence in Chapter 5. Baseline measurements were performed between 1987-1991 and cancer incidence was determined by linkage with cancer registries up to 1997. Complete baseline information was available for 33,796 persons of whom 140 developed lung cancer. Analyses were stratified to the histological types Kreyberg I and Kreyberg II (adenocarcinoma) tumors. Vegetable consumption was inversely associated with lung cancer incidence; adjusted RRs (95% CI) for quartiles of frequencies of intake were 1; 0.90 (0.59-1.37); 0.63 (0.39-1.03); 0.56 (0.33-0.95), respectively; p trend 0.01. This relationship was very strong for incidence of adenocarcinomas (p trend 0.003), whereas incidence of Kreyberg I tumors was not statistically significantly associated with vegetable intake (p trend 0.24). After adjustment for potential confounders, fruit consumption was not related to lung cancer, mainly due to adjustment for smoking. In conclusion, a higher vegetable intake was

associated with a lower lung cancer risk, especially of adenocarcinomas, whereas fruit consumption was not related to lung cancer in this study.

The association between cancer risk and adherence to the recommendation for fruits and vegetables, addressing both the aspect of quantity and variety, was examined in a prospective cohort study among 730 Dutch elderly men (Chapter 6). Follow-up for 10 years resulted in 138 cancer cases. The quantity of fruits and vegetables was assessed using a dietary history, while the variety in intake was based on a food frequency questionnaire. Adherence to the recommended amounts of fruit and vegetables was inversely associated with total cancer risk: the adjusted RR was 0.56 (95% CI 0.31-1.00). Eating the recommended daily 200 grams of vegetables was not related to cancer incidence, whereas eating the recommended 200 grams of fruit was associated with a 38% lower risk compared to eating less than 100 grams. Variety in vegetable intake was inversely associated with total cancer and with non-lung epithelial cancer: the RRs for the highest tertiles were 0.64 (95% CI 0.43-0.95) and 0.51 (95% CI 0.27-0.97), respectively. Adherence to the guidelines for fruit and vegetable intake was associated with lower cancer risk in this elderly male population. Besides quantity, also variety in intake was of importance.

Because accurate assessment of consumption of fruits and mainly of vegetables is difficult, plasma carotenoid concentrations were examined as a tool to categorize the usual vegetable and fruit intakes of 591 Dutch men and women aged 20-59 years in Chapter 7. Vegetable, fruit and juice consumption was assessed by a food frequency questionnaire (FFQ). In this sample of the general Dutch population, plasma β-cryptoxanthin was the best indicator for fruit intake. Because the variation in fruit intake contributed most to the variation in the total intake of vegetables, fruits and juices, β-cryptoxanthin also best indicated this total intake. Lutein best divided low and high vegetable consumers. Concentrations of carotenoids, individual or in combinations, could however not differ between all different quartiles of intake as assessed by the FFQ. Therefore, it was concluded that plasma carotenoid concentrations are only crude indicators of vegetable and fruit intake.

Fruit and vegetable consumption and the changes in this consumption during the period 1987/88-1997/98 were described for the Dutch population 19 years and older based on the National Food Consumption Surveys (Chapter 8). In 1997/98, the vegetable intake (g/d; mean ± SD) was 142 ± 102 in men and 138 ± 96 in women. For fruits, these figures were 105 ± 122 and 122 ± 121, respectively. Mean levels were below recommended intakes. In the 10-year period, the mean fruit plus vegetable consumption (excluding juices) decreased with 34 g/d (12%) in men and 23 g/d (8%) in women. Fruit and vegetable consumption was lowest and decreased

most in this period in the Dutch aged 19-35 with a low level of education. Given the evidence from observational studies, we estimated for this group the potential preventable cancer incidence over a 40-year period by increasing the fruit and vegetable intake according to several scenarios using a computer simulation model. The maximum theoretically reduction in cancer incidence, that is when all would consume the recommended 400 g/d, was estimated to be 14 to 22%.

In Chapter 9, results described in this thesis were summarized and methodological, etiological and public health issues were discussed. When studying the relationship between fruit and vegetable intake and epithelial cancer risk in prospective observational studies, valid assessment of fruit and mainly of vegetable intake, residual confounding by smoking, and experiencing enough power (regarding range of intake and number of cancer cases) to study the relation are major methodological concerns. Associations observed in recent cohort studies between fruit and vegetable intake and cancers of the lung, stomach and colon/rectum were weaker compared to earlier risk estimates. Part of this difference may be due to the fact that the majority of the earlier studies used a case-control design. In addition, differences in studied fruits and vegetables, in cancer types including histological types, over time, between geographical areas or other differences in populations studied may have clouded the picture. Taken all evidence together, an inverse association between fruit and vegetable intake and cancers of the lung, stomach and colon/rectum is still indicated. Based on the total evidence, we estimated the relative risks for these associations at approximately 0.75, 0.80 and 0.85, respectively, for consuming at least 400 grams of fruits and vegetables daily compared to less than 100 g/d. There is not enough evidence to point at specific fruits and vegetables or components of them as responsible actors.

Future observational studies need enough power to further investigate these associations and to refine risk estimates. They should pay attention to the shape of the association and explore the use of absolute risk estimates. Such studies need more valid measurements of usual fruit and vegetable intake, presumably by combining questionnaires and biological markers, and intermediate markers of cancer. Mechanistic studies, including the role of genes, should be continued to improve understanding of the carcinogenesis and how fruits and vegetable may interact in this process. Moreover, the role of viruses in the cancer process deserves more attention and improving immune function may be an aid in addition to lifestyle changes to reduce or postpone the risk of epithelial cancer.

Samenvatting

Epidemiologische studies hebben een inverse relatie tussen groente- en fruitconsumptie en epitheliale tumoren laten zien. Het ging hierbij voornamelijk om patiënt-controle onderzoeken, waarvan de resultaten kunnen zijn vertekend door selectie- en informatiebias. Het is de vraag of deze bevindingen te reproduceren zijn in prospectieve studies.

In dit proefschrift zijn prospectieve onderzoeken naar de relaties tussen consumptie van groente, fruit en in mindere mate andere plantaardige voedingsmiddelen en het risico op epitheliale tumoren beschreven. De voornaamste onderzoeksvraag hierbij was of een hogere inneming van groente en fruit gerelateerd was met een lager risico op epitheliale tumoren, met name longkanker. Vervolgens werd onderzocht of zulke inverse associaties toegeschreven konden worden aan subgroepen van of componenten in plantaardige voedingsmiddelen. Ook de variatie in groente- en fruitconsumptie is bestudeerd in relatie tot het risico op kanker. Daarnaast is onderzocht of concentraties van carotenoïden in plasma kunnen worden gebruikt om personen in te delen naar hun gebruikelijke groente- en fruitconsumptie. De consumptie van groente en fruit in Nederland is beschreven voor volwassenen. Voor personen van 19 tot 35 jaar met een laag opleidingsniveau is tenslotte een schatting gemaakt van de potentiële reductie in kankerincidentie door het verhogen van de groente- en fruitconsumptie.

Consumptie van plantaardige voedingsmiddelen werd bestudeerd in relatie tot sterfte aan colorectaalkanker met gegevens van 12.763 mannen van middelbare leeftijd verdeeld over de 16 cohorten van de Zeven Landen Studie (hoofdstuk 2). In deze ecologische studie werden relatieve risico's (RR) voor de 25-jaars sterfte aan colorectaalkanker berekend voor een verschil van 10% in de gemiddelde inneming van plantaardige voedingsmiddelen en –stoffen. Met behulp van de opschrijfmethode werden rond 1960 voedselconsumptiegegevens verzameld bij subgroepen van deze cohorten. Informatie over de inneming van voedingsstoffen was bepaald door chemische analyses van voedingsmiddelen. Consumptie van het totaal aan plantaardige voedingsmiddelen, groente en fruit was niet gerelateerd aan het risico op colorectaalkanker. De inneming van voedingsvezel was invers geassocieerd met de sterfte aan colorectaalkanker (RR 0,89, 95% betrouwbaarheidsinterval (BI) 0,80-0,97); dit komt overeen met een 33% lager risico wanneer de vezelinneming met 10 g/d toeneemt. Inneming van vitamine B6 (RR 0,84, 95% BI 0,71-0,99) en α-tocopherol (RR 0,94, 95% BI 0,89-0,99) waren ook invers geassocieerd met het risico op colorectaalkanker. Deze innemingen waren

echter sterk gecorreleerd met de inneming van voedingsvezel. De inneming van voedingsvezel bleek de beste indicator voor dat deel van de plantaardige voeding dat relevant was voor het verlagen van het risico op colorectaalkanker op populatieniveau.

Een vergelijkbare ecologische analyse is uitgevoerd naar de relatie tussen de consumptie van plantaardige voedingsmiddelen en maagkanker in hoofdstuk 3. Hiervoor werden opnieuw de Zeven Landen Studie gebruikt en relatieve risico's berekend voor een verschil van 10% in de gemiddelde inneming. Fruitconsumptie was invers geassocieerd met het risico op maagkanker (RR 0,96, 95% BI 0,91-0,99), terwijl consumptie van geraffineerde graanproducten een positieve relatie liet zien met het risico (RR 1,07, 95% BI 1,03-1,12). Een lage consumptie van fruit hing echter sterk samen met een hoge consumptie van geraffineerde graanproducten. De overige groepen van plantaardige voedingsmiddelen waren niet gerelateerd aan het 25-jaars risico op sterfte aan maagkanker. Hoewel een hoge consumptie aan geraffineerde graanproducten het maagkankerrisico leek te verhogen, kan deze bevinding ook een afspiegeling zijn van een voeding met weinig fruit.

In hoofdstuk 4 werd de groente- en fruitconsumptie in relatie tot het 25-jaars risico op longkankersterfte bestudeerd in een cohort Europese mannen. Rond 1970 werd de voedselconsumptie van Finse, Italiaanse en Nederlandse mannen van 50-69 jaar gemeten. Complete informatie was beschikbaar voor 3.108 mannen, van wie er 1.578 rookten aan het begin van de studie. Onder rokers was consumptie van fruit invers geassocieerd met longkankersterfte: gecorrigeerde relatieve risico's (RR) waren 0,56 (95% BI 0,37-0,84) en 0,69 (95% BI 0,46-1,02) voor respectievelijk het middelste en hoogste tertiel van inneming. Deze associatie was alleen statistisch significant in het Nederlandse cohort: RRs 0,33 (95% BI 0,16-0,70) en 0,35 (95% BI 0,16-0,74), respectievelijk. Analyses opgesplitst naar de intensiteit van sigarettenroken (niet, licht en zwaar) lieten alleen een inverse associatie voor zware rokers zien. Groenteconsumptie was niet gerelateerd aan het longkankerrisico bij rokers. In deze prospectieve analyse bij Europese rokende mannen was de consumptie van fruit invers gerelateerd met longkankersterfte. Deze associatie was beperkt tot zware sigarettenrokers.

In hoofdstuk 5 werd de relatie tussen groente- en fruitconsumptie en longkankerincidentie bestudeerd bij Nederlandse mannen en vrouwen van 20-59 jaar. De metingen aan het begin van het onderzoek werden verricht tussen 1987-1991 en informatie over de kankerincidentie werd verkregen door koppeling met kankerregistraties tot aan 1997. Complete informatie was beschikbaar voor 33.796 personen van wie er 140 longkanker kregen tijdens follow-up. Analyses werden

opgesplitst naar de histologische Kreyberg I en Kreyberg II (adenocarcinomen)-tumortypen. Groenteconsumptie was invers geassocieerd met longkankerincidentie: gecorrigeerde relatieve risico's (95% BI) voor kwartielen van de consumptiefrequentie waren respectievelijk 1; 0,90 (0,59-1,37); 0,63 (0,39-1,03); 0,56 (0,33-0,95); *p* trend 0,01. Dit verband was sterk voor de incidentie van adenocarcinomen (*p* trend 0,003), terwijl de incidentie van Kreyberg I-tumoren niet statistisch significant geassocieerd was met groenteconsumptie (*p* trend 0,24). Na correctie voor potentiële verstorende variabelen bleek fruitconsumptie niet gerelateerd te zijn aan longkanker, vooral tengevolge van de correctie voor rookgedrag. Concluderend kan gesteld worden dat een hogere groenteconsumptie geassocieerd was met een lager longkankerrisico, vooral van adenocarcinomen, terwijl fruitconsumptie geen relatie met longkanker liet zien.

Het verband tussen kankerrisico en het voldoen aan de aanbevelingen voor de consumptie van groente en fruit, zowel gericht op de hoeveelheid als de variatie, werd onderzocht in een prospectief cohortonderzoek bij 730 oudere Nederlandse mannen (hoofdstuk 6). Na 10 jaar bleken 138 van deze mannen kanker te hebben ontwikkeld. De geconsumeerde hoeveelheid groente en fruit werd gemeten met behulp van een dietary history, terwijl de variatie in consumptie werd vastgesteld met een voedselfrequentievragenlijst. Het voldoen aan de richtlijn voor de hoeveelheid groente en fruit was invers geassocieerd met het risico op kanker: het gecorrigeerde relatieve risico bedroeg 0,56 (95% BI 0,31-1,00). Het consumeren van de aanbevolen 200 gram groente per dag was niet gerelateerd met kankerincidentie, terwijl consumptie van de aanbevolen 200 gram fruit per dag samenhing met een 38% lager risico vergeleken met een consumptie van 100 gram per dag. Variatie in groenteconsumptie was wel invers geassocieerd met totaal kanker en met andere epitheliale tumoren dan longkanker: de relatieve risico's voor de hoogste tertielen van inneming waren respectievelijk 0,64 (95% BI 0,43-0,95) en 0,51 (95% BI 0,27-0,97). Het gebruik van groente en fruit volgens de aanbevelingen was geassocieerd met een lager risico op kanker in deze populatie van oudere mannen. Naast hoeveelheid speelde ook de variatie in consumptie een rol.

Omdat het moeilijk is om de consumptie van fruit, maar met name van groente valide te meten, is onderzocht of concentraties van carotenoïden in plasma gebruikt konden worden om 591 Nederlandse mannen en vrouwen van 20-59 jaar in te delen naar hun gebruikelijke groente- en fruitconsumptie (hoofdstuk 7). De consumptie van groente, fruit en sappen werd gemeten met een voedselfrequentievragenlijst. In deze steekproef uit de Nederlandse bevolking bleek plasma β-cryptoxanthin de beste indicator voor fruitconsumptie te zijn. Omdat de variatie in fruitconsumptie het meest bijdroeg aan de variatie in de totale consumptie van groente, fruit en sappen, was

plasma β-cryptoxanthin ook de beste marker voor deze totale consumptie. Luteïne was het best in staat personen met een lage en een hoge groenteconsumptie te onderscheiden. Carotenoïdenconcentraties, zowel individueel als in combinaties, konden niet worden gebruikt om de verschillende consumptiekwartielen zoals gemeten met de voedselfrequentievragenlijst te onderscheiden. De conclusie is daarom dat plasma carotenoïden slechts globale indicatoren van groente- en fruitconsumptie zijn.

In hoofdstuk 8 zijn op grond van de voedselconsumptiepeilingen de groente- en fruitconsumptie en de veranderingen daarin gedurende de periode 1987/88-1997/98 beschreven voor de Nederlandse bevolking van 19 jaar en ouder. In 1997/98 bedroeg de groenteconsumptie (gemiddelde ± SD) van mannen 142 ± 102 g/dag en van vrouwen 138 ± 96 g/dag. Voor fruitconsumptie waren deze cijfers respectievelijk 105 ± 122 en 122 ± 121. Deze gemiddelde niveaus lagen onder de aanbevolen hoeveelheden. In de periode van 10 jaar nam de gemiddelde groente- en fruitconsumptie (exclusief sappen) af met 34 g/dag (12%) bij mannen en 23 g/dag (8%) bij vrouwen. Groente- en fruitconsumptie was het laagst en nam in deze periode het meest af bij Nederlanders van 19 tot 35 jaar met een laag opleidingsniveau. Op basis van resultaten van observationele studies hebben we voor deze groep een schatting gemaakt, gebruikmakend van een computersimulatie, van de kankerincidentie die potentieel te voorkomen is gedurende 40 jaar door het verhogen van de groente- en fruitconsumptie volgens een aantal scenario's. De maximale theoretische reductie in kankerincidentie, dat wil zeggen wanneer iedereen de aanbevolen hoeveelheid van 400 g/dag zou consumeren, werd geschat op 14 tot 22%.

In hoofdstuk 9 zijn de resultaten van dit proefschrift samengevat en methodologische, etiologische en public health aandachtspunten bediscussieerd. Bij het bestuderen van de relatie tussen groente- en fruitconsumptie en epitheliale tumoren zijn valide metingen van de consumptie van fruit maar met name van groente, residuele confounding door roken en voldoende power om de relatie te bestuderen (betreffende de range van consumptie en het aantal kankergevallen) belangrijke methodologische aandachtspunten. De associaties die gevonden zijn in recente cohortstudies voor de relatie tussen groente- en fruitconsumptie en kanker van de long, maag en colon/rectum, waren zwakker in vergelijking met eerdere risicoschattingen. Een deel van dit verschil kan mogelijk worden verklaard doordat de meerderheid van de eerdere onderzoeken patiënt-controle studies waren. Daarnaast kunnen ook verschillen in de bestudeerde soorten groente en fruit, in de soorten kanker inclusief histologische typen, in de tijd, tussen geografische gebieden en andere verschillen tussen onderzochte populaties het beeld hebben vertroebeld. De totale beschikbare

informatie duidt echter nog steeds op een inverse relatie tussen groente- en fruitconsumptie en kanker van de long, maag en colon/rectum. Op basis van tot nu toe beschikbare gegevens zijn relatieve risico's voor deze associaties geschat op respectievelijk ongeveer 0,75, 0,80 en 0,85 voor het consumeren van tenminste 400 g/dag groente en fruit vergeleken met een consumptie van minder dan 100 g/dag. Er is echter onvoldoende bewijs om specifieke groente- en fruitsoorten of stoffen daarin verantwoordelijk te stellen voor deze bescherming.

Toekomstige observationele studies dienen voldoende power te hebben om deze associaties verder te onderzoeken en de risicoschattingen te verfijnen. Deze onderzoeken zouden aandacht moeten besteden aan de vraag of het verband tussen groente- en fruitconsumptie en kankerrisico lineair, log-lineair of anders van vorm is. Tevens zouden zij het gebruik van absolute risicoschattingen moeten exploreren. Zulke studies hebben valide metingen van de gebruikelijke groente- en fruitconsumptie nodig, waarschijnlijk te bereiken door een combinatie van het gebruik van vragenlijsten en biologische merkers voor de inneming van voedingsstoffen, en intermediare merkers voor kanker. Mechanistische studies, inclusief de rol van genetische gevoeligheid, zouden moeten worden uitgevoerd om het inzicht in de carcinogenese en hoe groente en fruit in dit proces kunnen ingrijpen, te vergroten. Verder verdient de rol van virussen in het kankerproces meer aandacht en zou het verbeteren van de immuunfunctie bestudeerd dienen te worden als mogelijke manier om naast veranderingen in voeding en leefstijl het risico op epitheliale tumoren te verminderen of het optreden hiervan uit te stellen.

Dankwoord

Promoveren mag dan soms een eenzaam gebeuren lijken, allerlei mensen hebben op één of andere manier bijgedragen aan de totstandkoming van dit boekje. Graag dank ik een ieder hartelijk hiervoor! Een aantal personen verdient op deze plaats een persoonlijke noot.

Beginnend met begeleiders. Daan, jouw enthousiasme voor onderzoek is geweldig om te ervaren. Hoe vaak ik niet van je heb gehoord dat iets 'fascinerend' is, is niet te tellen. Het ontrafelen van 'het geheim van Kreta' is volledig aan je toevertrouwd. Ik heb bewondering voor het feit dat je in je drukke functie deze fascinatie vasthoudt en de tijd vindt om manuscripten zo snel en grondig van commentaar te voorzien. Met name in de laatste fase, toen jij je sabbatical leave had, heb je geweldig meegewerkt aan de afronding van mijn proefschrift. Hiervoor dank! Frans, als tweede promotor had je misschien wat meer afstand tot het eigenlijke werk. Onze samenwerking gaat echter al langer terug. Zo rond mijn afstuderen kwam ik bij je binnenlopen en mocht blijven. Toen is het begonnen, met de groente en fruit! De mate waarin je mensen kunt motiveren heb ik altijd erg gewaardeerd. Daarnaast heb je tijdens mijn promotie-onderzoek op cruciale momenten de rol van intermediair geweldig vervuld, waarvoor dank. En tenslotte Bas, mijn co-promotor. Nauwgezet worstelde je je door mijn manuscripten en kwam met de meest mooie engelse frasen op de proppen. Door je integere houding werden alle facetten zorgvuldig gewogen. Dank je voor je inzet en voor je persoonlijke belangstelling.

Graag wil ik de deelnemers aan de onderzoeken waarvan ik de gegevens heb kunnen gebruiken bedanken. Slechts enkelen van hen (in Zutphen en Velika Krsna) heb ik in levenden lijve mogen ontmoeten; maar zonder jullie geen onderzoek! Ook de co-auteurs van de verschillende hoofdstukken, mijn hartelijke dank voor de bijdragen. Met name Marga, jouw kundigheid was een grote hulp! Hier ook een plaatsje voor Maria van Hemert-Janssen. Het was afzien met die rietjes uit de -196°C, maar het is toch maar mooi allemaal gelukt! Laurence Frank en Annemieke van der Waal, om jullie als studenten begeleid te hebben was gezellig en ik dank jullie hartelijk voor het werk dat jullie hebben verzet.
Tal van mensen boden ondersteuning door middel van hun secretariële, automatiserings- dan wel statistische werkzaamheden. De goede zorgen van Els en Anke in Bilthoven, en van Marie ('die andere mevrouw Jansen') in Wageningen zorgden voor de juiste afstemming tussen de agenda's van al 'die heren'. Hans, Jan en Ruud in Bilthoven, en Dirk en Ben in Wageningen waren de 'computermannen'

die onontbeerlijk zijn voor epidemiologisch onderzoek. Ook Nico, Hendriek en Cor waren broodnodig voor het aanpakken van statistische dan wel SAS-problemen. Bedankt allemaal voor jullie hulp maar vooral voor de prettige samenwerking!

En dan natuurlijk al mijn (andere) collega's! Ik zou niet zonder kunnen! Met zo'n samenwerkingsproject en het reeds beginnen met een nieuwe baan heb ik ze in ruime aantallen (gehad). Alle (oud-)collega's, bedankt voor jullie gezelligheid en belangstelling. Die borrels, etentjes, feestjes en trips maakten van de promotie-tijd een hele gezellige tijd! De RIVM-collega's speelden hierin wel de grootste rol, en dan met name Claudia, Angelika, Saskia, Tommy, Peggy, Ina, Jantine, Jeanne, Susan, Ilja, Jolanda, Caroline, Sandra, Rob, Edith en Marianne. We moeten in de toekomst regelmatig wat afspreken hoor! En dan wil ik graag Claudia en Angelika nogmaals noemen. Bedankt dat jullie zulke geweldige kamergenoten waren! Jullie brachten gezelligheid en vrolijkheid maar ook zeker hulp en steun als dat nodig was. Ik vind het heel fijn dat jullie mij als paranimfen willen bijstaan.
Ook (oud-)collega's uit Wageningen wil ik bedanken voor hun gezelligheid en belangstelling, vooral tijdens mijn verblijf in John Snow die later 'dagjes Wageningen' werden. Hierbij wil ik met name Annemien, Marijke, Edine, Liesbeth, Juul, Marie-Françoise, Judith, Mariska, Ingeborg, Dorien, Frouwkje, Esther, Petra, Louise en Hélène noemen. Nynke, jou zet ik even apart. Je bent namelijk meer dan een oud-collega! Ik vind het erg leuk dat het er naar uitziet dat jij mijn promotie toch mee gaat maken en dat jullie in de buurt komen wonen!
En dan natuurlijk de 'nieuwe' collega's bij TNO Voeding. Jullie belangstellende en motiverende opmerkingen heb ik zeer gewaardeerd. Twee personen wil ik graag in het bijzonder noemen. Karin, lieve en zorgzame kamergenoot, en Sandra, het enthousiasme waarmee je me coacht is geweldig en werkt aanstekelijk!

Bedankt lieve familie en vrienden. Eigenlijk vond ik een promotie 'gewoon werk'; niet iets waar je als familie en vrienden nou zo'n grote rol in hebt. Maar ondertussen hebben jullie mij met je belangstelling en gezelligheid toch elke keer weer energie gegeven om vol goede moed verder te gaan.
Koos, mijn proefschrift wil ik graag aan jou opdragen. Jouw sterke doch lieve persoonlijkheid is een voorbeeld voor velen. De kans dat je jouw plannen voor een proefschrift verwezenlijkt lijken klein, daarom wil ik je graag betrekken in het mijne. Ook Carla, mijn moeder, wil ik hier graag bedanken voor haar zorg en belangstelling. En nu jij lieve Mark, al vind je dat promoveren maar een poppenkast. Ik kan geen dankwoord schrijven zonder jou te noemen. Jij grote relativerende factor met je 'wiederholungs-grappen'. Ik heb er wel zin in om met jou een lekkere stek te maken van ons nieuwe huis! En reisplannen hebben we nog genoeg! Een dikke zoen voor jou om je te bedanken voor wie je voor me bent.

About the author

Margje Carola Johanna Francisca Jansen was born on July 15 1970 in Nijmegen, The Netherlands. After completing secondary school in 1988 (VWO at the Maurick College in Vught) she studied Human Nutrition at Wageningen University. As part of this study she conducted a research project at the Radboud Hospital Nijmegen, spent a practical training period at the University of Newcastle, Australia, and performed two market research projects. In August 1994, she received her MSc degree.

In August 1994, she was appointed at the Department of Epidemiology and Public Health of the Wageningen University. There she worked on a desk research as rationale for the national campaign on fruits and vegetables, followed by a national survey into the lifestyle and risk factors for coronary heart disease. In April 1996, she was appointed at the Department of Chronic Diseases Epidemiology of the National Institute of Public Health and the Environment (RIVM) in Bilthoven as research associate. In October 1996, she started her PhD-project described in this thesis. This project was conducted at the Department of Chronic Diseases Epidemiology of the RIVM in collaboration with the division of Human Nutrition and Epidemiology of the Wageningen University. She participated in the educational program of the Graduate School VLAG (Food Technology, Agrobiotechnology, Nutrition and Health Sciences). In 1997, she attended the Annual New England Epidemiology Summer Program at Tufts University, Boston, USA. She joined the Public Health training for PhD students of the Netherlands School of Public Health, and participated in the European Nutrition Leadership Program of 2000 in Luxembourg. She collaborated on the update of the desk research on the relation between fruits and vegetables and chronic diseases. Moreover, she worked on a project under the authority of the Commission 'Trends Food Consumption' of the Dutch Health Council to model health effects following changes in dietary consumption.

Since October 2000, she works as project manager at the Department of Nutritional Epidemiology of TNO Nutrition and Food Research in Zeist, The Netherlands.

Financial support by Wageningen University for the publication of this thesis is gratefully acknowledged. TNO Nutrition and Food Research, the Fruit and Vegetable Information Bureau, and the Graduate School NIHES are thanked for their additional financial support.