Do fermented dairy products and calcium protect against colorectal cancer ?

contributions from epidemiology

PROEFSCHRIFT

ter verkrijging van de graad van doctor
aan de Rijksuniversiteit te Maastricht,
op gezag van de Rector Magnificus, Prof. Dr. H. Philipsen,
volgens het besluit van het College van Dekanen,
in het openbaar te verdedigen
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om 14.00 uur

door

Ellen Kampman

'Here is Edward Bear, coming downstairs
now, bump, bump, bump, on the back
of his head, behind Christopher Robin. It is, as
far as he knows, the only way of coming
downstairs, but sometimes he feels that there
really is another way, if only he could stop
bumping for a moment and think of it'

(from Winnie-the-Pooh, A.A. Milne)

voor mijn ouders

Contents

Chapter 1

Introduction

Introduction

Colorectal cancer contributes considerably to the incidence of cancer in the Netherlands, like in most westernized countries. Each year a malignant tumor of the colon or rectum is newly diagnosed in about 7,000 Dutch men and women.[1] Colorectal cancer is the third most common cancer among men, after cancer of the lung and the prostate, and the second most common among women, behind breast cancer.[1] Although new methods of surgery, chemotherapy and radiotherapy are being developed and applied, mortality rates of colorectal cancer remain relatively high. New developments in treatment are not expected to markedly increase survival rates in the near future, which makes prevention of this type of cancer even more important.

A role of dietary factors in colorectal carcinogenesis seems biologically plausible and is also widely recognized. Changes in dietary patterns may provide important clues in colorectal cancer prevention. Both experimental research and analytical epidemiology provide possibilities to unravel the various aspects of a westernized diet involved in the aetiology of colorectal cancer. The results of these studies, however, have not yielded clues for preventive measures that can be implemented easily in the population at large.

Like a diet rich in vegetables and fruits, a high consumption of fermented dairy products, such as yogurt, buttermilk and cheese, might decrease colorectal cancer risk. Fermentation of dairy products with lactic acid bacteria is an ancient method of preservation, and for centuries several of these bacteria species have been believed to contribute to the prevention and treatment of gastro-intestinal disturbances. Already in 1908, Eli Metchnikoff developed a theory that lactic acid bacteria in the digestive tract could prolong life.[2] More recently, a protective effect on tumor development has been suggested for these specific dairy products.[3,4] This hypothesis mainly derives from experimental research, as epidemiological studies considering the effect of fermented dairy products on cancer are scanty. In addition to their content of lactic acid bacteria, fermented dairy products are an important source of calcium. Experimental and epidemiological studies suggest a protective effect of calcium in colorectal carcinogenesis. However, the results of epidemiological studies are still controversial and a higher intake of this nutrient is not generally recommended yet.

Chapter 2 of this thesis provides an overview of current knowledge on the aetiology of colorectal cancer and summarizes results of experimental and epidemiological studies on the associations between consumption of fermented dairy products, intake of calcium and colorectal cancer risk which have been published before and during the studies included in this thesis.

In most epidemiological studies, the consumption of dairy products and calcium is just one of the many dietary factors studied. The specific aim of the research described in this thesis was to study whether the consumption of fermented dairy products and the intake of calcium is inversely associated with the risk of colorectal cancer and thus might become tools in its prevention. The thesis includes four epidemiological studies described in Chapters 3 through 8, which differ with respect to disease end-point, study design, methods of exposure assessment and study population.

Chapter 3 describes results of two ongoing prospective studies at the Harvard School of Public Health in Boston, US: the Nurses' Health Study and the Health Professionals Follow-up Study. The analyses focus on the association with colorectal adenomatous polyps, which are considered to be precursors of colorectal cancer.

Chapters 4 through 7 describe the results of a case-control study on colon cancer conducted in the Netherlands between 1990 and 1993. This study was intentionally conducted in the Netherlands, since the consumption of fermented dairy products is traditionally integrated in the Dutch food pattern. This case-control study is one of the first population-based case-control studies in the Netherlands in which the Cancer Registry was successfully used for case recruitment; furthermore, an alternative procedure of control recruitment was applied. These aspects of design and conduct are discussed in detail in *Chapter 4*. In *Chapter 5* the main hypothesis is tested, while *Chapters 6 and 7* deal with other life-style and dietary factors which were additionally explored.

Finally, *Chapter 8* describes the association between fermented dairy products, calcium and colorectal cancer as studied prospectively in the ongoing Netherlands Cohort Study on diet and cancer.

Chapter 9 integrates the results of the four studies and reviews the strengths and limitations of the studies. Moreover, results are discussed in the context of previously published epidemiological and experimental studies.

Chapters 3, 5, 6, 7 and 8 are included in this thesis in the form in which they have been published, accepted or submitted for publication in international journals.

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Chapter 2

Aetiology of colorectal cancer

Contents

Current concepts

Fermented dairy products and calcium: a hypothesis

Current concepts

Proliferation, adenoma and carcinoma formation

The classical view of carcinogenesis included two stages: initiation, which involved mutation of the DNA, and promotion, in which expansion of initiated cells occurred. Nowadays, carcinogenesis is considered to be a multistage process, involving 5 or more different changes.[1] In the past decades, several of the different stages in colorectal cancer development are more precisely identified. It is believed that tumorigenesis of colorectal adenocarcinomas proceeds through mucosal cell hyperproliferation to adenoma formation, growth, and malignant transformation.[2] Studies on a inherited greatly predisposing condition for colon cancer, Familial Adenomatous Polyposis (FAP), confirm this hypothesized pathway. In FAP patients as well as disease free relatives, an increased thymidine labelling index, expansion of the proliferative zone, surface labelling and acceleration of migration rate in the flat mucosa between existing polyps and tumors as well as in the mucosa prior to polyp formation have been identified.[3,4] Increased colonic epithelial cell proliferation has also been observed in sporadic colorectal cancer cases.[3]

It has been observed that a high proportion of large adenomas contain focal areas of malignancy, and a high proportion of early colorectal tumors contain remnants of adenomatous tissue.[5] It has been suggested that all colorectal carcinomas have a precursor adenoma stage, although most adenomas do not progress to malignancy and remain benign.[5] Adenomatous polyps antedate the clinical diagnosis of colorectal cancer by an average of approximately ten years.[5]

Whereas adenomas are evenly distributed along the large intestine,[2] carcinomas are more likely to develop in the sigmoid colon or rectum. Large adenomas are also more common in the sigmoid colon, suggesting that factors that cause the initial formation of an adenoma differ from those that cause its subsequent growth.[2]

The risk of developing an adenoma might depend on both genetic and environmental factors, with the former determining which members of a uniformly exposed population are at greatest risk of developing the disease and the latter determining the proportion of the high-risk component of the population that actually does so.[5]

Changes on the molecular level

Vogelstein and colleagues have provided extensive evidence that there is an accumulating series of chromosomal and genetic alterations that accompany the transition from the normal colonic mucosa to carcinoma.[6] According to this model (Figure 2.1), the accumulation of at least four or five genetic changes are required

to produce a malignant tumor, their order not being of critical relevance.[6] Several different genetic alterations which occur during colorectal tumorigenesis have been identified, involving activation of oncogenes coupled with the inactivation of tumor suppressor genes.
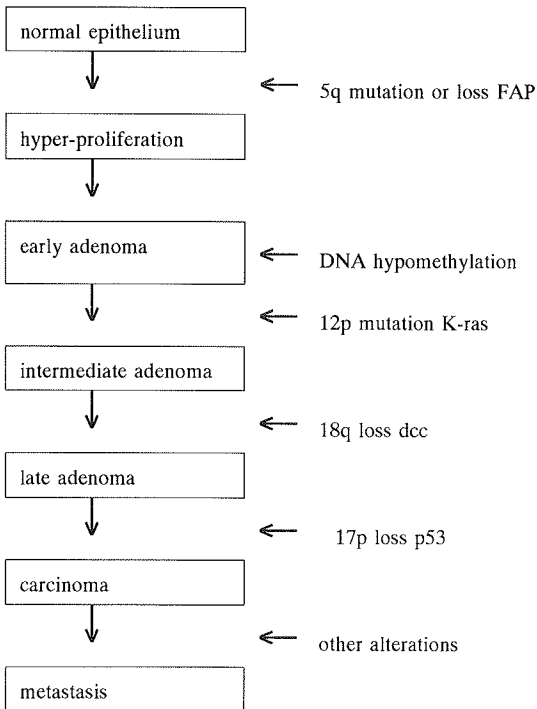
Figure 2.1   Hypothesized genetic model for colorectal tumorigenesis according to Fearon and Vogelstein; the accumulation of changes rather than their order is considered relevant in this model for determining the tumor's biological properties.[6]

Although the order of genetic changes is not critical, a given order might be more frequently observed. One of the earliest genetic changes accompanying tumor progression is a significant loss of methyl groups in DNA.[7] The role of this hypomethylation is still unclear. It has been suggested that folate deficiency induces hypomethylation.[8,9]

Mutation of the ras oncogene was found in less than 10% of early-stage adenomas and in approximately 50% of intermediate-stage adenomas, late-stage adenomas and colon carcinomas.[10] Three different ras-genes have been isolated: Ha-ras, Ki-ras and N-ras. Ki-ras mutations are most common.[11] The Ki-ras gene encodes for a G-protein (p21) which may be involved in signal transduction.

Mutations in this gene may lead to uncontrolled cell growth.[11] Ki-ras mutations can be induced by methylating carcinogens.[11]

Allelic deletions were discovered in chromosomes 5, 17 and 18, which might harbour tumor-suppressor genes. Deletions on the short arm of chromosome 17 (17p) are common and occur in more than 75% of colorectal adenomas. These deletions appear to be relatively late events and are associated with the transition from the benign adenoma to the malignant carcinoma.[12] The allelic loss on chromosome 17 has been shown to be the p53 gene, which codes for a phospho-protein involved in the control of cell proliferation.[13] It is speculated that a mutated p53 gene in a colorectal carcinoma can provide selective growth advantage by promoting tumor progression even in the presence of a normal p53 allele.[14] P53 mutations are also common in other cancers, as breast, lung and stomach cancer.[15] Exogenous and endogenous mutagens influence the type and site of the p53 gene base substitutions.[16]

Allelic losses on chromosome 18 are also common and occur in less than 10% of small and intermediate stage adenomas,[14] 45% of large adenomas and in approximately 75% of colorectal carcinomas, which suggest that these events occur before p53 alterations. The deletion site (dcc=deleted in colorectal carcinomas) on chromosome 18q has been identified as a gene coding for a cell-adhesion molecule, mediating cell growth and differentiation.[17]

Allelic deletion in chromosome 5q has been found in almost 40% of colorectal carcinomas and sporadic adenomas studied.[18] The tumor suppressor gene (mcc = mutated in colorectal carcinomas) is believed to be connected with G-protein activation, which is important in transducing signals in the cell. Additional studies are needed to elucidate the relationship between mcc gene mutations and sporadic colorectal cancers and to determine whether mcc is mutated in familial adenomatous polyposis coli families.[14]

The above is a reflection of current insight. The rapid developments in this area require regular updating.

The role of diet and life-style

Environmental factors are suggested to be the driving forces behind the transformation of the normal colonic mucosa to cancer, among these, diet and life-style are considered to be highly important.[19] These factors influence the content of the colonic lumen, the enzymatic activity of the colonic flora and consequently the exposure of the epithelial cells to carcinogenic or promoting substances.[20-22] Several nutritive and non-nutritive components of our diet might be involved.[23,24]

One of the relatively old, well-established hypotheses suggests an elevated risk as a consequence of our westernized high fat, low fiber diet.[23,24]

Biological and -more recently- molecular explanations have been proposed. The 'physiological' explanation for this elevated risk is the bile acid/volatile fatty acid hypothesis. Dietary fat increases the bile acid and fatty acid excretion in the colonic lumen.[25] The microbial flora hydrolyses the polar glyco and tauro conjugate bile acids to form secondary bile acids in the faeces.[5] Secondary bile acids in their ionized form and free fatty acids may damage the colonic epithelium, stimulating cell proliferation.[26-29] Two bacterial enzymes have been proposed to be important in the colonic bile-acid metabolism: 7-dehydroxylase and 4-5 nuclear dehydrogenase, which are more frequently present in populations with a high risk of colorectal cancer.[30] The activity of these enzymes is not favoured by acidic conditions.[30]

Moreover, it has been suggested that the colonic flora converts free fatty acids in diacylglycerol (DAG) which is an activator of protein kinase C, inducing cell replication signals.[31]

Dietary fiber binds bile acids and free fatty acids, increases stool bulk, and can be fermented to volatile fatty acids. These may be directly anti-carcinogenic[32] and by lowering pH may reduce the conversion of primary to secondary bile acids as well.[33]

In order to explain the role of a high fat/low fiber diet on the molecular level, these dietary factors have been viewed as indicators for carcinogenic and anti-carcinogenic substances. Vegetables and fruits are not only important sources of fiber, but also contain vitamin C, carotenoids, phenols, flavonoids and indoles, with a variety of anti-carcinogenic properties.[34,35] Vegetable consumption has been consistently shown to be inversely associated with colon cancer risk in epidemiological studies, though inverse associations with fruit consumption have been less frequently observed.[35,36] Howe and colleagues completed a meta-analyses of 13 case-control studies and concluded that a higher overall fiber intake decreases colon cancer risk.[37] A high fat diet might be a source of arylamines from meat proteins[38] and cancer promoters resulting from high temperature cooking.[39] In ecological studies both a high fat and a high meat diet have been correlated with colorectal cancer risk.[23] As reviewed by Potter et al.,[40] analytical epidemiological studies on fat, protein and meat consumption show, almost consistently, a positive association with risk of colorectal cancer.

In addition to this 'established' theory, other factors which have been suggested to exert an effect on colorectal cancer risk are physical activity, alcohol consumption, parity (see hormonal factors), use of nonsteriodal anti-inflammatory drugs, cholecystectomy, caffeine and smoking.

Physical activity stimulates colon peristalsis and decreases random, nonpropulsive segmentation activity.[41] The resulting shortened stool transit time and decrease in segmentation-induced mixing may give putative fecal carcinogens less contact with

the colonic mucosa. In epidemiologic studies, physical activity has been consistently associated with an increased risk of colon cancer.[40] Total energy intake is often considered to be an acceptable proxy for physical activity. In most prospective studies, no association with total energy has been found. In most case-control studies, an elevated risk has been observed with increasing energy intake, but this might be attributed to a general overreporting of the cases.

The mechanism by which <u>alcohol</u> entails an increased risk for colorectal cancer is not clear. One hypothesis involves its effects on lipid secretion and metabolism. Moderate alcohol intake decreases cholesterol saturation of bile by increasing bile acid concentration.[42] Longnecker and colleagues conducted a meta-analysis on alcohol and colorectal cancer including 27 epidemiologic studies, and concluded that because the magnitude of the association between alcohol and risk of colorectal cancer was small, the findings regarding a causal role of alcohol were inconclusive.[43]

<u>Non-steroidal anti-inflammatory drugs</u> inhibit the synthesis of prostaglandins which might play a role in cell proliferation, neoplasia and immune response.[44]

<u>Cholecysectomy</u> might increase colon cancer risk by the alteration of the bile acid secretion that follows after removal. A meta-analyses on 33 case-control and 6 cohort studies conducted by Giovannucci and colleagues, showed a significant positive association between cholecystectomy and risk of colorectal cancer from the case-control data, while the results from cohort studies were generally null. Time since cholecystectomy appeared to be important. Although risk varied by study design and in most studied time since cholecystectomy was not reported, the authors conclude that some characteristics of bile-acid metabolism increases the risk of cancer of the proximal colon.[45]

<u>Caffeine</u> might increase serumcholesterol.[46] If the mechanism leading to higher cholesterol levels involves a reduction in the excretion of bile acids, coffee consumption might reduce the risk of colorectal cancer. However, in his review of the epidemiologic literature Rosenberg concludes that although some studies suggest an inverse association, 'the data are not compelling and a biological mechanism is not established'.[47]

The physiological mechanism by which <u>smoking</u> might increase colon cancer risk is unclear. One possibility might be via the production and swallowing of compounds such as arylamines. Although several epidemiological studies suggest an association between smoking and colorectal adenoma risk, only recently Giovannucci and colleagues reported a significant positive association with colorectal cancer in the Nurses Health Study and the Health Professionals Follow-up study.[48,49] They point to the fact that the induction period between the start of smoking and the diagnosis of cancer is important in this respect and might be at

Hormonal influences

Although colon cancer is essentially the only cancer that occurs with approximately equal frequency in men and women, the rates tend to show an excess among women before the age of 50 years and an excess among men after that age, as reviewed by Potter and colleagues.[40] Subsite risk has also been shown to vary by sex: women have higher rates of right-sided carcinoma.[40] Possibly, there are sex differences in etiology, which may be explained by environmental and or hormonal factors.[50] In 1980, McMichael and Potter proposed that higher parity, early age at first birth, and use of oral contraceptives would each be associated with a reduced risk of colon cancer as a result of changes in lipids and bile acids that occur with changes in the hormonal milieu.[51] Sex hormones might also moderate cell proliferation and differentiation other than by intraluminal sterol metabolism.[50]

Most case-control studies confirm an inverse association between parity and colorectal cancer risk, while the studies of other reproductive factors such as age at first birth, age at menarche and age at menopause are more limited and less persuasive.[50] The review by Potter et al. indicated that the results of prospective studies do not suggest an association with parity and age at first birth.[40] The authors suggest that it is only in older women that higher parity is associated with reduced risk, which is comparable to the suggested association between parity and breast cancer risk.[40]

Both case-control and cohort studies do not consistently support a substantial reduction of colon cancer risk with the use of exogeneous hormones.[40]

Another observation which might explain differences in colorectal cancer risk between men and women is that gender-related differences in gut metabolism exists. Men and women show, under controlled experimental conditions, differences in large bowel function (stool bulk, transit time, volatile fatty acid production) and bile acid excretion.[52]

Fermented dairy products and calcium: a hypothesis

Besides the 'established' risk factors, relatively new hypotheses concern the potential protective role of fermented dairy products[53] (e.g. yogurt, buttermilk, cheese) and calcium[54] in colorectal carcinogenesis.

Ecological studies

Associations between colorectal cancer incidence or mortality and national consumption of milk or milk products have been reported by several investigators. Alder and McKeown-Eyssen reviewed these ecological data[55] and concluded that 6

out of 12 studies conducted before 1988 observed a positive association, while the other half reported a negative association. All studies reporting a negative association controlled for dietary fat intake in the analysis. Their own ecological study among 38 countries, using food disappearance data[56] suggests an inverse association between the availability of total dairy products and colon cancer mortality, adjusting for animal fat and cereal fiber intake (r = −0,36, p < 0.05). The inverse relationship with dairy product intake in the 38-countries study was mainly attributable to two of the countries included, i.e. Finland and Iceland.[57] In Finland, the intake of dairy products, non-fermented as well as with Lactobacilli fermented milk products is highest in the world. In a cross-sectional study in Scandinavia comparing various risk factors in men from a high risk (Copenhagen) and low risk area (rural Finland), representing a threefold difference in colon cancer incidence, the intake of milk and dietary fiber was higher in low risk areas, whereas no difference in saturated fat intake was observed.[58] A higher number of faecal Lactobacilli were counted among the low-risk Fins as compared to the high-risk Danish men.[58] However, in an extension of this study including two additional study areas with intermediate incidences (rural Denmark and Helsinki), the association with milk and milk product intake did not remain significant.[59] As the intake of milk products in Denmark is relatively high as well, the difference in intake between the Finnish and Danish may not be high enough to observe an association. In a study by Benno et al.[60] higher numbers of fecal lactobacilli were found in Japanese at low risk of colon cancer compared to Canadians at high risk.

As previous ecological studies did not specifically address <u>fermented dairy products</u>, we investigated the correlation between per capita consumption of fermented milk and the incidence of colon cancer among 16 countries. In these analyses, fermented milk intake per capita in 1981[61] was not correlated with colon cancer incidence[62] perse, but after adjusting for % energy from fat a negative correlation (r = −0.49, p = 0.05) was demonstrated (Figure 2.2). When Finland was excluded from these analyses, the correlation did not remain significant.

Dairy products contribute significantly to daily <u>calcium</u> intake. In the Netherlands 70% of calcium intake is from dairy sources. It has been suggested from ecological as well as experimental studies that a protective effect of dairy products in general may be attributed to the calcium content of dairy products.[54] In the US the intake of calcium is lowest in the north-eastern states where colon cancer mortality is highest.[63] However, in the 38-countries study, no association with calcium was observed.[55]

In general, one should be careful interpreting results from ecological studies as the assessment of intake and incidence data is hardly comparable between countries. Furthermore, since data necessary to control for confounding at an individual level

are not available, results from these international correlations require confirmation by individual based analytical epidemiology.[64]
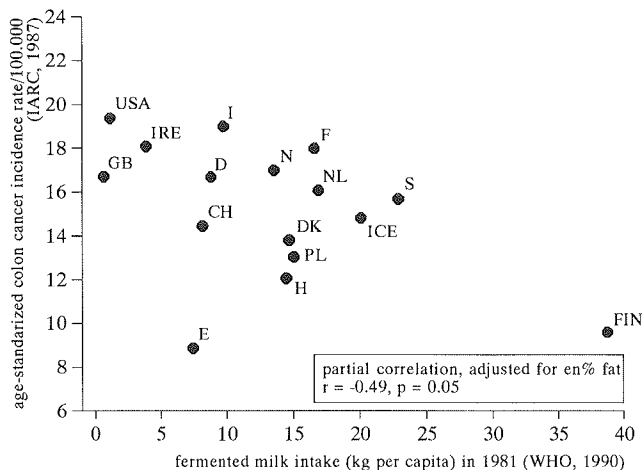
Figure 2.2   Per capita consumption of fermented milk consumption and colon cancer incidence.

Analytical epidemiological studies

In contrast to ecological studies, case-control and cohort studies provide the possibility to adjust for potential confounding variables.

As we started our investigation on the association of fermented dairy products, calcium and colorectal cancer in 1990, results from other comparable studies conducted before and in or after 1990 will be discussed in chronological order.

Table 2.1a shows the main characteristics and results of 13 <u>case-control studies</u> <u>conducted before 1990,</u> which addressed the relationship between the consumption of (fermented) dairy products, calcium intake and colorectal cancer or polyp risk. Two retrospective studies addressed the intake of <u>fermented milk</u> (yogurt and buttermilk).[65,66] An inverse association with fermented milk and colon cancer was observed in a American study conducted by Young and Wolf (OR = 0.65, 95% CI = 0.41-1.01),[65] while a Japanese study observed a positive non-significant association with yogurt (OR = 1.52 (ns)).[66] In the American study an inverse association with <u>cheese</u> consumption was found (OR = 0.72, 95% CI = 0.51-1.05),[65] which was also not confirmed in the Japanese study (OR = 1.36 (ns)).[66] A French case-control study mentioned a non-significant inverse association with cheese consumption (OR = 0.9) for colorectal cancer,[67] and a non-significant positive association (OR = 1.2) for colorectal polyps.[68] In a Belgium study an OR of 1.0 was

found for cheese consumption.[69]

For total dairy products, Slattery and colleagues[70] observed a significant negative association in Utah, while an Australian[71] and an American study[72] observed weak non-significant negative associations and a study in Greece describes a weak non-significant positive association.[73]

Approximately, one third of the case-control studies included in Table 2.1a support an inverse association with milk or calcium. In the study conducted by Slattery et al. a significant negative association with colon cancer was observed.[70] Kune and colleagues observed a non-significant decreased risk with high milk, high calcium consumption after adjustment for many confounding variables.[71] Non-significant inverse associations were observed in three other studies for colon cancer[67,74,75] and in one other for rectal cancer.[66] Miller et al.[76] and Manousos et al.[73] both observed slightly elevated risk associated with high milk consumption. After adjustment for fat intake, this association was not longer significant. A non-significant positive association with calcium was also observed in the Belgian study by Tuyns et al.[77]

Prospective cohort studies conducted before 1990 were not conclusive either (Table 2.2a). One of the four studies conducted addressed the consumption of cheese, which intake was significantly positively associated with colon cancer death in men, and non-significantly inversely associated with colon cancer death in women.[78] In the same study, milk consumption was inversely associated with colon cancer death in men.[78] However the number of deaths in the reference category was too small to draw firm conclusions.

Calcium was found to be significantly inverse associated in one of these four studies. In this study by Garland and colleagues,[79] two dietary histories were obtained one year apart from a cohort of approximately 2,000 men who were followed for a period of 19 years. The risk of colorectal cancer was found to be about three times higher in men who consumed the lowest levels of calcium compared to those with the highest consumption and a dose-response relationship was observed. In contrast, two other prospective studies by Heilbrun et al.[80] and Wu et al.[81] did observe no association and a non-significant inverse association respectively. In both studies no association with fat intake was observed. None of the prospective results addressed colorectal polyps.

After 1989, while we were conducting our study, another 12 case-control and 4 prospective studies reported their results on dairy products, calcium and colorectal cancer. One large case-control study by Peters and colleagues on colon cancer addressed the consumption of yogurt: an odds ratio of 0.8 per 10 grams of yogurt was observed assuming a log-lineair dose-response, and adjusted for dietary calcium.[82]

Table 2.1a. Dairy products, calcium, colorectal polyps and colorectal cancer: 13 case-control studies 1975-1989.

First author, year [ref]	Population	No. of cases/ endpoint	Controls/source	Dietary method	Risk estimates (OR) highest vs lowest intake	Calcium intake (or contrast) among controls (mg/day)	Adjusted for
Miller, 1983 [76]	Canada	542 colorectal cancer	535 hospital and neighbourhood	diet hist	milk: $OR_{C,M}$ = 1.2 (ns) $OR_{C,F}$ = 1.3 (ns) $OR_{R,M}$ = 1.1 (ns) $OR_{R,F}$ = 1.2 (ns)	nm	age, saturated fat
Manousos, 1983 [73]	Greece	100 colorectal cancer	100 hospital	diet hist (i=80)	milk and milk products: OR = 1.2 (0.6, 2.4)	nm	–
Pickle, 1984 [72]	US, Nebraska	86 colorectal cancer	176 hospital	diet hist (i=57)	dairy products: OR_C = 0.74 (ns) OR_R = 0.92 (ns)	nm	age, sex, ancestry, usual residence
Tajima, 1985 [66]	Japan	93 colorectal cancer	186 hospital	ffq	yogurt: OR_C = 1.5 (ns) OR_R = 1.7 (ns) cheese: OR_C = 1.4 (ns) OR_R = 1.2 (ns) calcium: OR_C = 1.1 (ns) OR_R = 0.5 (ns)	nm	age, sex
Macquart-Moulin, 1986 [67]	France, Marseilles region	399 colorectal cancer	399 hospital	diet hist (i=158)	cheese: OR = 0.9 (ns) milk: OR = 0.7 (ptr<0.05) calcium: OR = 0.7 (ns)	nm	age, sex, calories, weight
Macquart-Moulin, 1987 [68]	France, Marseilles region	223 colorectal adenoma and 29 villous polyps	238 hospital	diet hist (i=158)	cheese: OR = 1.2 (ns) milk: OR = 0.9 (ns) calcium: OR = 1.2 (ns)	median: men = 831 women = 772	age, sex, calories, weight
Tuyns, 1987 [77]	Belgium	818 colorectal cancer	2,851 population	diet hist	calcium: OR_C = 1.3 (ns) OR_R = 1.0	mean: 700	age, sex, province, calories
Tuyns, 1988 [69]	Belgium	818 colorectal cancer	2,851 population	diet hist	milk: OR_C = 1.8(ptr<0.0001) OR_R = 1.7(ptr<0.01) cheese: OR_C = 1.0 OR_R = 1.0		age, sex, province

Kune, 1987 [71]	Australia, Melbourne	715 colon cancer	727 population	diet hist (i=300)	milk products:
					  OR = 0.9 (0.6, 1.3)
					  OR_M = 1.0
					  OR_F = 0.8 (0.5, 1.3)
					milk drinks:
					  OR = 0.9 (0.6, 1.2)
					  OR_M = 1.4 (0.9, 2.2)
					  OR_F = 0.5 (0.3, 0.8)
					calcium: OR = 0.8 (0.6, 1.1)
					  OR_M = 1.1 (0.7, 1.7)
					  OR_F = 0.6 (0.4, 1.0)
						<597,
						>1150 (men)
						
						<528,
						>1043 (women)
							unknown

Graham, 1988 [74]	US, West. New York State	428 colon cancer	428 hospital and neighbourhood	diet hist	estimate not given calcium: ns	nm	age, education, phosphorus, fat

Slattery, 1988 [70]	Utah	231 colon cancer	391 population	qffq (i=99)	dairy products:
					  $OR_{C,M}$ = 0.4 (0.2, 0.8)
					  $OR_{C,F}$ = 0.5 (0.2, 1.1)
					calcium: $OR_{C,M}$ = 0.4 (0.2, 0.9)
					  $OR_{C,F}$ = 0.5 (0.3, 1.0)
						≤641,
						>1,402 (men)
						
						≤592,
						>1,141 (women)
							age, religion, fiber, calories

Young, 1988 [65]	US, New York	353 colon cancer	618 population	diet hist	cultured milk: OR = 0.7 (0.4, 1.0) cheese: OR = 0.7 (0.5, 1.1) milk: OR = 1.1 (0.8, 1.5)	nm	age, sex, age*sex

Lee, 1989 [75]	Singapore Chinese	203 colorectal cancer	425 hospital	qffq	milk: OR_C = 0.8 (0.5, 1.3)
					  OR_R = 1.1 (0.6, 2.1)
					calcium: OR_C = 0.9 (0.5, 1.5)
					  OR_R = 0.8 (0.4, 1.5)
						mean:
						men: 338 ± 177
						women: 356 ± 194
							age, sex, Chinese dialect group, occupation

C = colon cancer or polyps; R = rectal cancer or polyps; F = female; M = male; ptr = p for trend; nm = not mentioned; (q)ffq =(semi quantitative) food frequency questionaire; i = number of items; diet hist = dietary history.

Table 2.1b. Dairy products, calcium and colorectal polyps and colorectal cancer: 12 case-control studies 1990-1993.

Author, year	Population	No. of cases/ end point	Controls/ source	Dietary method	Risk estimates (OR) highest vs lowest intake	Calcium intake (or contrast) among controls (mg/day)	Adjusted for
Negri, 1990 [83]	Italy	910 colorectal cancer	1,032 hospital	ffq (i=29)	milk: OR = 1.0 (0.7, 1.3) cheese: OR_C = 1.1 (0.8, 1.4) OR_R = 1.3 (1.0, 1.8) calcium: OR_C = 1.1 (0.8, 1.6) OR_R = 1.2 (0.8, 1.9)	nm	age, sex, education, residence, pasta, rice, meat, green vegetables, coffee
Benito, 1990 [90]	Majorca	286 colorectal cancer	499 population and hospital	ffq	dairy products: OR_C = 1.07 (ns) OR_R = 3.08 (ptr <0.01) OR_M = 4.30 (ptr <0.05) OR_F = 1.66 (ptr <0.05)	nm	age, sex, weight, SES, physical activity, no. meals per day, cereals, potatoes, meat, vegetables, eggs
Whittemore, 1990 [85]	China and Chinese in US	905 colorectal cancer	2,488 population	diet hist (i=84)	calcium: OR_C < 1.0 (ns) OR_R < 1.0 (p <0.01)	nm	age, sex, saturated fat
Benito, 1991 [91]	Majorca	286 colorectal cancer	499 population and hospital	ffq	calcium: OR = 1.48 (ns)	nm	–
Kune, 1991 [92]	Australia, Melbourne	49 colorectal adenoma ≥ 1 cm	727 population	diet hist (i=300)	milk drinks: OR_M = 3.7 (1.5, 9.5) OR_F = 0.7 (0.2, 2.0)	nm	nm
Peters, 1992 [82]	US, Los Angeles	746 colon cancer	746 population	qffq (i=139)	yogurt: OR per 10 grams = 0.8 (0.7, 1.0) calcium: OR = 0.4 (0.3, 0.7)	1,049 ± 504	age, sex, (calcium), sources of calories, family history, weight, physical activity, (pregnancy history)
Bidoli, 1992 [84]	North-eastern Italy	248 colorectal cancer	699 hospital	diet hist	milk: OR_C = 1.0 OR_R = 1.0 cheese: OR_C = 1.4 (ptr=0.17) OR_R = 1.6 (ptr=0.08)	nm	age, sex, ses, bread, polenta, eggs, tomatoes

Iscovich, 1992 [88]	Argentina, La Plata area,	110 colon cancer	220 neighbour-hood	diet hist (i=140)	dairy products: OR = 3.0 (1.2, 7.6) whole milk: OR = 1.6 (0.8, 3.1) (uv) cheese: OR = 1.9 (0.9, 4.0) (uv)	nm	education, bmi, meat, fish, eggs, vegetables
Zaridze, 1993 [86]	Russia, Moscow, Khabarovsk	217 colorectal cancer	217 population and neighbour-hood	diet hist	calcium: OR = 0.5 (0.2, 1.1) milk: OR = 0.5 (0.3, 1.0)	men: < 747,9, >1,477,9 women: <890,4, >1,545,1	energy intake, education
Meyer and White, 1993 [87]	US, Washington State	424 colon cancer	414 population	ffq (i=71)	calcium: OR_M = 1.1 (0.9, 1.4) OR_F = 0.7 (0.6, 1.0)	median: 896	age, interviewer, calories, alcohol, fiber
Steinmetz and Potter, 1993 [89]	Australia Adelaide	220 colon cancer	438 population	ffq (i=141)	dairy foods: OR_M = 1.1 (0.6, 2.2) OR_F = 1.6 (0.8, 3.4)	nm	age, sex, occupation, bmi, alcohol, (age at first live birth)
Benito, 1993 [93]	Spain, Majorca	101 colorectal adenoma	242 population	ffq	dairy products: OR = 1.2 (ptr >0.05) cheese: OR = 0.7 (ptr >0.05) calcium: OR = 0.9 (ptr=0) milk: OR = 1.0 (ptr >0.05)	median cases and controls: 725	age, sex, calories, physical activity, rural residence

C = colon cancer or polyps; R = rectal cancer or polyps; F = female; M = male; ptr = p for trend; nm = not mentioned; (q)ffq =(semi quantitative) food frequency questionaire; i = number of items; diet hist = dietary history.

Table 2.2a. Dairy products, calcium and colorectal cancer: 4 prospective studies 1975-1989.

First author,year [ref]	Population	Follow-up (years)	No. of cases, endpoint/ cohort	Dietary method	Risk estimates (RR) highest vs lowest intake	Calcium intake (or contrast) cohort (mg/day)	Adjusted for
Garland, 1985 [79]	US Mid West	19	49 male colorectal cancer/ 1,954	28d-diet hist (2x)	calcium: $RR_M = 0.3$ p < 0.05	328/ 1,000 kcal	age, smoking, bmi, other dietary variables
Phillips, 1985 [78]	US California	21	182 colorectal cancer deaths/ 25,493	ffq (i=21)	cheese: $RR_{C,M} = 1.9$ (1.0-3.6) ptr < 0.05 $RR_{C,F} = 0.8$ (0.5-1.4) $RR_R = 1.0$ (0.5-2.2) milk: $RR_{C,M} = 0.5^*$(0.2-1.1) ptr > 0.05 $RR_{C,F} = 1.1$ (0.5-2.2) $RR_R = 1.2$ (0.6-2.7)	nm	age, (sex)
Heilbrun, 1986 [80]	Hawaii Japanese	13-16	99 male colorectal cancer/378 controls (nested case-control)	24h-recall	calcium: $RR_M = 0.7$, p > 0.05 (in high phosphate $\geq$ 1,032, high fat $\geq$ 61 group)	median: 501	age
Wu, 1987 [81]	US, Los Angeles	4.5	126 colorectal cancer/ 11,888	ffq (i=56)	calcium: $RR_M = 0.9$ (0.4-1.7) $RR_F = 0.9$ (0.5-1.6)	nm	age

C = colon cancer or colon polyps; R = rectal cancer or rectal polyps; F = female; M = male; * = based on small number of deaths; ptr = p for trend; nm = not mentioned; (q)ffq = (semi quantitative) food frequency questionaire; i = number of items; diet hist = dietary history.

Table 2.2b. Dairy products, calcium and colorectal cancer: 4 prospective studies 1990-1993.

First author,year [ref]	Population	Follow-up (years)	No. of cases, endpoint/ cohort	Dietary method	Risk estimates (RR) highest vs lowest intake	Calcium intake (or contrast) cohort (mg/day)	Adjusted for
Stemmermann, 1990 [94]	US, Japanese	19-22	323 male colon cancer/7,472	24h-recall	calcium: RR_M = 0.6 (0.4-0.9) (sigmoid) RR_M = 0.8 (0.6-1.1) (total colon)	age-adjusted mean non-cases: 503	age
Ursin, 1990 [95]	Norway	11.5	155 colorectal cancer/ 15,914	ffq	milk: RR = 0.9 (ns)	nm	age, sex, place of residence
Willett, 1990 [131]	US	6	150 female colon cancer/ 88,751	qffq (i=61)	estimates not given cheese ns whole milk ns calcium ns	nm	unknown
Bostick, 1993 [96]	US, Iowa	4	212 female colon cancer/ 35,216	qffq (i=127)	calcium: RR_F = 0.7 (0.4-1.0) calcium diet: RR_F = 1.0 (0.6-1.6) calcium suppl.: RR_F = 0.7 (0.4-1.0) dairy products: RR_F = 0.7 (0.4-1.4)	total calcium: 1,119 ± 3 dietary calcium: 835 ± 2	age, calories, height, parity, vitamin E, total vitamin E*age, low-fat meat

C = colon cancer or colon polyps; R = rectal cancer or rectal polyps; F = female; M = male; * = based on small number of deaths; ptr = p for trend; nm = not mentioned; (q)ffq = (semi quantitative) food frequency questionnaire; i = number of items; diet hist = dietary history.

Cheese consumption was addressed in two Italian case-control studies:[83,84] non-significant positive associations were found.

In about half of the studies which mentioned milk and/or calcium intake, inverse associations were observed.[82,85-87] An important observation was the clear dose-response effect of calcium in the large US case-control study by Peters et al.[82] In another US case-control study an inverse association with calcium intake was observed for women only, for men a non-significant positive association was observed.[87] In a relatively small Argentinean study, a non-significant positive association was observed for whole milk consumption, while the intake of dairy products in general significantly increased risk.[88] Positive associations with the intake of dairy foods or calcium and colon or rectum cancer risk were observed in two other studies.[89,90,91]

Two case-control studies on colorectal polyps were conducted. The results of a small study in Australia indicated a significant positive association with milk drinks among men, while in women a non-significant inverse association was found.[92] An Italian study found no important association with dairy product, milk or calcium intake.[93] In the same study, a non-significant inverse association with cheese consumption was found.[93]

None of the prospective studies published after 1989 addressed fermented dairy products. In three of the four prospective studies addressing milk and/or calcium, an inverse association was observed.[94-96] In the study conducted by Stemmermann et al. in a Japanese Hawaiian population using a 24h recall method the association with calcium and sigmoid cancer was significant (RR = 0.6, 95% CI = 0.4-0.9).[94] For the colon as a whole this association was less strong and not significant (RR = 0.8). In the most recent study published, the Iowa Women Health Study, calcium from supplements (mean intake among non-cases:283 mg/day) was associated with risk (RR = 0.66, 95% CI = 0.43-1.02), while only a very weak inverse association was observed with calcium from dietary origin (RR = 0.95, 95% CI = 0.57-1.61) (mean intake among non-cases: 835 mg/day).[96]

The diversity in findings among case-control as well as prospective studies may be partially explained by differences in populations, differences in methodology and differences in the bacterial strains used to ferment dairy products. Most studies are conducted among populations with a relatively low and homogeneous dairy product intake. Only a few studies addressed the interactions with dairy products, calcium and other nutrients as fat or fiber, or focused on different stages of tumor development as the outcome or stratified disease according to subsites within the colon.

Experimental studies

Lactic acid bacteria

Diet affects the composition of the intestinal flora, and carcinogenic substances derived from food may be produced by bacteria within the colon.[21,22] It has been suggested that lactic acid bacteria, which belong to the normal intestinal microflora, contribute to the stabilization of the bacterial flora and maintain resistance against pathogens.[97]

Since the oral administration of commercial and simulated yogurt containing *Lactobacillus bulgaricus and Streptococcus thermophilus* inhibited initial Ehrlich ascites tumor growth in mice[98] and supplements with *Lactobacillus acidophilus* delayed the occurrence of DMH-induced colon cancer in rats,[99] several short-term animal and human experimental studies have been conducted studying the mechanisms by which suppletion of lactobacilli might exert anti-carcinogenic properties.[97]

Three different mechanisms have been suggested (see Table 2.3). Lactobacilli might modulate the colonic flora, thereby influencing enzyme activity and reducing the conversion of procarcinogens to carcinogens in the colon. The activity of specific bacterial enzymes is elevated by a high meat, western-type diet.[53] Supplementation of the human diet with milk containing viable *L. acidophilus* of human origin, which is bile resistant, caused a significant decline in faecal bacterial enzymes supposed to be involved in the carcinogenic process, such as β-glucuronidase, 7-α-dehydroxylase, nitroreductase and azoreductase.[53,100] It is not clear whether ingested lactobacilli reduce enzyme activity due to implantation of the viable strain or to suppression of enzyme activity in other intestinal bacteria by viable and unviable strains.[101]

The second hypothesized mechanism involves a stimulation of the immune respons, locally as well as systematically. Oral administration of milk containing *L. bulgaricus* or *L. casei* to mice[102] was shown to activate lymphocytes and macrophages. Addition of yogurt to human peripheral blood lymphocyte cultures stimulated by a mitogen (Con-Canavalin A)[103] increases the production of γ-interferon, a cytokine which has anti-proliferative properties and has been able to activate Natural Killer cells.

Thirdly, in vitro studies have shown that lactic acid bacteria have the ability to absorb cooked food mutagens.[104] Human experimental studies have shown that intake of *L. acidophilus* significantly reduced mutagen excretion after consumption of fried meat.[105]

Table 2.3   Mechanisms suggested for the protective effect of fermented dairy products on colorectal cancer risk.

First author, year [ref]	Human/animal	Strain	Observations
Modulation colonic flora			
Ayebo, 1980 [132]	healthy humans	*L. acidophilus*	β-glucuronidase −(3:
Goldin, 1984 [53]			nitroreductase − (2x)
Marteau, 1990 [100]			azoreductase − (1x)
	colon cancer patients		β-glucosidase − (1x)
Lidbeck, 1991 [133]			
Immunology			
Perdigon, 1986 [102]	mice	*L. acidophilus*	macrophages +
De Simone, 1986 [103]	human lymphocytes	*L. bulgaricus*	γ-interferon +
Mutagen absorption			
Zhang and Ohta, 1991 [104]	in vitro	*L. acidophilus, B. bifidum, S. cremoris*	food mutagen binding capacity +
Lidbeck, 1992 [105]	healthy humans	*L. acidophilus*	faecal mutagen binding capacity +

+ increase; − decrease.

   For Lactobacilli to exert an effect on the intestinal microflora, survival through the intestinal tract might be important. Robins-Brown and Levine[106] have shown that *L. acidophilus* and *L. bulgaricus*, when taken with milk, have the ability to pass through the stomach in equal numbers. Jejunal fluid samples were taken from volunteers at varying intervals and lactobacilli were cultured on a selective medium. *L. acidophilus* was recovered from more samples and in higher counts that *L. bulgaricus*. When given 500 ml *L. acidophilus* fermented milk per day, within one week the number of lactobacilli increased markedly in human volunteers.[107,108] However, one week after supplementation was stopped, the numbers of lactobacilli had decreased to almost the same levels as before administration. This implies that colonization is not established and consumption of lactobacilli might need to be at least very frequent to exert an effect.

   Although promising, experimental research on lactic acid bacteria and fermented dairy products is preliminary.

Calcium

Several biological mechanisms have been suggested for a protective effect of calcium in colon carcinogenesis. Calcium[109] or -more recently- calciumphosphate,[110] might reduce colorectal cancer risk by binding bile acids and free fatty acids, thereby diminishing the proliferative stimulus of these compounds on the colon mucosa. This hypothesis has been supported by animal experiments.[109,111,112] An alternative hypothesis, based on in vitro studies in human epithelial cells,[113-116] proposes that calcium might inhibit the proliferation of colonic epithelial cells directly by inducing differentiation. Furthermore, administration of calcium reduced chemical carcinogen-induced tumor yield in rodents,[117-119] and reduced Ki-ras G to A mutations in colorectal neoplasms in rats.[120] For calcium to influence cell proliferation directly, absorption into the cells is necessary. Vitamin D increases calcium absorption. Experimental studies suggest inhibition of cell proliferation by vitamin D, which might be due to vitamin D itself or its effect on calcium absorption.[40]

Before 1990, 4 out of 6 small trials, which were not placebo-controlled, have shown that supplemental calcium reduces cell proliferation in man (Table 2.4a).[113,114,116,121] Lipkin and Newmark reported the first trial in 10 persons with a family history of colon cancer.[113] Calcium (1250 mg administered as calciumcarbonate) was added to their normal diets for a period of three months and rectal biopsies were obtained before and after supplementation. The average level of proliferation of epithelial cells in the colon was lower after calcium supplementation. However, since no control group was used, these changes could be attributable to temporal fluctuations in cell proliferation. Furthermore, it was unclear whether all subjects experienced a reduction in cell proliferation or whether this was confined to those individuals whose levels were initially the highest and who might therefore be expected to show reduced levels during a repeated examination through chance variation. In a small randomized, placebo-controlled trial no decrease in proliferation activity was noticed using the same method of proliferation assessment.[122]

The baseline intake of calcium, as assessed through dietary questionnaires, was similar among the studies and was usually slightly less than the adult recommended dietary allowance (RDA) of 800 mg/day. Doses of supplementation were comparable between studies and the tritiated thymidine method was used in all studies to assess the labelling index. The labelling index reflects the proliferative state of the mucosa: the more proliferating cells, the higher the labelling index. In the study by Gregoire and colleagues the duration of supplement use was shorter than in the other studies.[122] However, since the turnover rate of the rectal mucosa is a few days, a significant effect was demonstable after as little as one month.[123]

Table 2.4a Human experimental studies on calcium and distal colonic epithelial proliferation
1985-1989.

First author year [ref]	Design	Study population/ No. of subjects	Ca-diet mg/ day	Ca-supplement[*] dose g/day	duration /months	Method	Change in labelling index
Lipkin, 1985 [113]	uncon-trolled[**]	HNPCC/10	700	1.25	2-3	T[***]	–
Buset, 1986 [114]	uncontrolled	FCC/5	nm	1.5	1-2	T	–
Buset, 1986 [114]	uncontrolled	sporadic adenoma/3	nm	1.5	1-2	T	0
Rozen, 1989 [121]	uncontrolled	FCC and sporadic adenoma/35	nm	1.3-1.5	3	T	–
Lipkin, 1989 [116]	uncontrolled	HNPCC/21	700	1.3-1.5	3-4	T	–
Lipkin, 1989 [116]	uncontrolled	FAP/7	700	1.3-1.5	3-4	T	variable response
Gregoire, 1989 [122]	placebo-controlled	colonic resection/30	669	1.2	1	T	+ (p=0.06)

* Calcium carbonate unless otherwise indicated; ** Without a placebo control group;
*** Labelling index determined using tritiated thymidine; – decrease; + increase; 0 no effect;
FCC Family history of Colorectal Cancer; HNPCC Family members of Heriditary Non-
Polyposis Colon Cancer; FAP Familial Adenomatous Polyposis; nm = not mentioned.

An important striking difference between the trials mentioned was that the reported baseline labelling index in the trials by Lipkin and colleagues (16.7%, 12.1% and 9.1% respectively)[113] was twice as high as those reported by Gregoire et al. (4.9%)[122] and Rozen et al. (5.3%).[121]

After 1989, 7 different research groups publiced the results of 11 trials (Table 2.4b). In five trials a significant reduction in cell proliferation could be determined,[123-126] in two a non significant reduction was observed,[123] two did not observe any reduction[127,128] and in two an increase in proliferation was observed.[129] In the study by Barsoum et al.,[124] a stathmokinetic method examining colonic crypt cell production rate was used to assess cell proliferation. This method takes the cell cycle time, growth fraction and the crypt cell population into account.[124] Kleibeuker et al.[129] and O'Sullivan et al.[125] determined labelling index using 5-bromodeoxyuridine and immunohistochemistry. Both studies did not use a placebo-treated control group.

Table 2.4b Human experimental studies on calcium and distal colonic epithelial proliferation 1990-1993.

First author, year [ref]	Design	Study population/ No. of subjects	Ca-diet mg/day	Ca-supplement dose g/day	Ca-supplement duration /months	Method	Change in labelling index
Stern, 1990 [127]	randomized, placebo-controlled	FAP/31	1,102	1.2	9	T	0
Barsoum, 1992 [126]	placebo-controlled, double blind	adenoma/14	nm	1.3	2	C	–
Wargovich, 1992 [123]	randomized, placebo-controlled, single-blinded	previous sporadic adenoma/6	702	1.5	3	T	– (p=0.08)
Wargovich, 1992 [123]	randomized, placebo-controlled, single-blinded	previous sporadic adenoma/6	702	2.0	1	T	–
Wargovich, 1992 [123]	randomized, placebo-controlled, single-blinded	sporadic adenoma/6	729	2.0	1	T	–
Wargovich, 1992 [123]	randomized, placebo-controlled, single-blind	sporadic adenoma/6	729	2.0	1	T	– (p=0.06)
Bostick, 1993 [128]	randomized, double blind, placebo-controlled	sporadic adenoma/21	951	1.2	2	T	0
Kleibeuker, 1993 [129]	uncontrolled	adenoma/17	nm	1.5	3	B	+
Kleibeuker, 1993 [129]	uncontrolled	adenoma/7	nm	1.5	12	B	+
O'Sullivan, 1993 [125]	uncontrolled	adenoma/20	nm	1-2	1	B	–
Thomas, 1993 [126]	double blind placebo-controlled	adenoma/25	nm	1.5	6	C	–

* Calcium carbonate unless otherwise indicated; T = tritiated thymidine; B = 5-bromode-oxyuridine and histochemistry; C = colonic crypt cell production rate; – = decrease;
+ = increase; 0 = no effect; FAP = Familial Adenomatous Polyposis; nm = not mentioned.

Wargovich et al. experimented with different doses of supplementation.[123] They published two small controlled trials, in which a dose of 1.5 g/day resulted in an insignificant 20% decrease in the labelling index, whereas a dose of 2.0 g/day resulted in a 29% decrease which was significant.

In a double blinded controlled trial[128] with calcium supplementation of 1.2 grams a day, the difference between change in the mean Labelling Index from baseline to 8 weeks' follow-up in the placebo group versus the calcium group was not statistically significant. Kleibeuker et al.[129] report an increase of the labelling index after 3 months as well as one year of supplementation. This is the only study which used biopsies from the mid sigmoid, all other studies used rectal biopsies to determine cell proliferation.

During these past 5 years a considerable number of placebo-controlled trials on calcium and cell proliferation are conducted. The results were not conclusive, however, partially because the techniques used to study cell-proliferation and the preferable end-point studied are still discussed.[130]

Overall, it can be concluded that, despite the research activities on fermented dairy products and calcium as related to colorectal cancer risk, results remain contradictory and our studies still provide a relevant contribution.

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Abstract

A high intake of calcium, vitamin D or specific dairy products is thought to reduce the incidence of colorectal cancer. The association of these nutrients and foods with the occurrence of colorectal adenomas, precursors of cancer, was studied in two large US cohort studies.

Cases were patients with a diagnosis of adenomatous polyps of the left colon o rectum (331 men, 1986-1990; 350 women, 1980-1988) controls were persons with endoscopic findings negative for adenoma (9,159 men and 8,585 women).

After adjustment for age, total energy, family history of colorectal cancer, body mass index, alcohol consumption, folate, intake of saturated fat and fiber, indications fo endoscopy, and previous endoscopy, total calcium intake was not associated with adenoma (relative risk (RR), highest versus lowest quintile of intake: men, 1.13, 95% confidence interval (CI) 0.76-1.66; women, 1.17, 95% CI = 0.81-1.69).

Total vitamin D intake was unrelated to the risk for adenoma in men (RR = 1.29 95% CI = 0.87-1.93). An inverse nonsignificant association was observed in women in the 1980-1988 analyses (RR = 0.68, 95% CI = 0.41-1.13, trend p = 0.09), mainly attributable to the intake of multivitamin supplements, but an analysis of 4-year data (1984-1988) using a more detailed dietary assessment showed no association with vitamin D (RR = 1.04, 95% CI = 0.65-1.67).

Milk consumption and intake of total fermented dairy products were not related to adenoma risk.

In conclusion, the occurrence of colorectal adenoma was neither related to calcium intake nor to milk consumption, whereas vitamin D from supplements but not diet was slightly, but not significantly, inversely associated with risk among women only.

Introduction

It has been hypothesized that diets high in fat and low in dietary fiber increase the risk for colon cancer.[1] An increased risk has also been attributed to the consumption of red meat[1,2] and a decreased risk to the consumption of vegetables and fruits.[3] An additional hypothesis suggests a protective effect of calcium or the consumption of calcium-rich milk products.[4] Support for this relation derives from clinical and experimental studies[5-7] and from ecological data on colorectal cancer.[8-10] It has been hypothesized that calcium reduces the risk for colon cancer by forming insoluble soaps with ionized fatty acids and secondary bile acids in the colon lumen, potentially diminishing the proliferative stimulus of these substances on the colon mucosa.[5,6] A direct effect of calcium on the proliferative activity of the mucosa has also been suggested.[7] However, case-control and cohort studies of colorectal cancer are not conclusive; some studies observed an inverse association with calcium or milk consumption,[11-13] while others suggested only a slight non-significant association[14,15] or no relation.[16-18]

Other nutrients or products associated with calcium intake may also be important. For instance, a high phosphate/calcium ratio in the diet may modify the possible inverse association with calcium.[5] Epidemiologic[19,20] and experimental studies[21-23] suggest a distinct protective mechanism of vitamin D that possibly involves cell proliferation. Furthermore, apart from the calcium and vitamin D content of milk, a protective effect against tumor development has been suggested for fermented dairy products,[24-28] which contain micro-organisms that may influence the normal colonic flora[29-31] or activate the immune system.[32,33] Although in physiologic studies, only specific strains of viable microorganisms seem to influence tumor occurrence or growth,[34] recent epidemiologic studies in the United States suggest an inverse association between the consumption of yogurt and cheese and colon cancer.[35,36]

An effect of calcium or vitamin D on cell proliferation or an altered bacterial metabolism of fermented milk products may be particularly important for the early stages of tumor development represented by the formation and growth of colorectal polyps. Previous studies considering the association between calcium or milk consumption and colorectal polyps[37-39] have been limited by retrospective diet assessment. To our knowledge, no studies on colorectal adenomas have addressed the intake of vitamin D or fermented milk products other than cheese.

To study prospectively the associations of the intake of calcium, vitamin D and dairy products with the occurrence of colorectal adenoma in men and women, we used data from two large US cohort studies: the Health Professionals Follow-Up Study (HPFS) and the Nurses' Health Study (NHS). The associations of saturated fat, dietary fiber,

women, 659 (91%) were contacted, of which 593 (90%) confirmed they had a polyp
and gave permission for review of medical records. After 8 years of follow-up, 509
colorectal polyp cases were confirmed, 350 being adenomatous polyps of the distal
colon or rectum.

In total, the 4-year follow-up analyses of the HPFS included 9,490 men (251 with
adenomas of the descending/sigmoid colon, 80 with rectal adenoma and 9,159 noncases
or controls). The 8-year follow-up in the NHS, included 8,935 women (224 with
adenomas of the descending/ sigmoid colon, 126 with rectal adenomas, and 8,585
controls).

Because the 1984 questionnaire provided more detailed information, for some
analyses we also assessed the occurrence of polyps in women between 1984 and 1988,
using the 1984 questionnaire as the baseline. The 1984-1988 analyses included 7,271
women (180 with adenomas of the descending/sigmoid colon and 103 with rectal
adenomas). Most of the subjects in the 1984-1988 follow-up were also in the 1980-
1988 follow-up (99%), the exceptions being the small number of women who
completed a valid food-frequency questionnaire in 1984 but not in 1980.

Data analysis
The nutrients of interest were analyzed using quintiles of intake, according to the
distribution in the studied population. Calcium and vitamin D intake were considered
with and without the intake of supplements, and from both dairy and non-dairy origin.
The prevalence rate ratio, based on the prevalence of polyps detected at endoscopy,
was used to calculate the relative risk. Relative risks (RR) were calculated using the
lowest quintile of intake as the reference and 95% confidence intervals were calculated
between the high and low quintiles. We tested for overall trends by using the median
of each quintile of calcium or vitamin D intake as a continuous variable.

The consumption of individual dairy products was studied by grouping the foods
into four or five frequency categories, based on the distribution of intake of the product
in the population at large. Relative risks and 95% confidence intervals were calculated
comparing participants who consumed a particular food with those who reported a
frequency of less than once a month. We tested for trend by modeling the reported
frequencies as a continuous variable.
The consumption of fermented milk products combined was studied using quintiles of
the summed frequencies of consumption of each product (yogurt, cottage cheese, hard
cheese, sour cream) weighed by the standard portion sizes or calcium content.

To control for several potentially confounding and modifying variables
simultaneously, we used multiple logistic regression models including age (in seven
categories), total energy intake, history of colon cancer in either parent, indication for

endoscopy, endoscopies before the study period, body mass index (in quintiles), smoking (never, past, current), physical activity and quintiles of intake of saturated fat, dietary fiber, alcohol, and folate. Since the time of polyp occurrence is not known and the outcome is the prevalence of polyps at endoscopy, we used logistic regression analysis instead of a prospective approach. Because the study population was selected by endoscopy, we adjusted for indication of endoscopy. To study the possible effect modification of the intake of saturated fat, vitamin D and phosphorus, we assessed the association with calcium (in tertiles) within tertiles (low, medium, high) of these nutrients. A high phosphorus intake relative to the intake of calcium was evaluated using quintiles of the calcium/phosphorus ratio with the lowest quintile as the reference. Similar analyses were conducted separately for cases with left colon and rectal polyps and for cases with small (< 1 cm) and larger (≥ 1 cm) polyps. When there was more than one polyp, the size of the largest was considered.

Results

Population characteristics

Table 3.1 presents the characteristics of the male and female study population and the prevalence of possible risk factors for colorectal adenoma, according to intake of calcium and vitamin D. Men and women with the highest intake of calcium or vitamin D tended to be a little older at endoscopy, had higher intakes of dietary fiber, protein and phosphorus, a lower intake of alcohol, and smoked less. Men and women in the highest quintiles of calcium and vitamin D intake more often reported having undergone an endoscopy before the study period than persons with the lowest intake. About 26% of the male and 16% of the female cases (34% of the 1984-1988 follow-up population) had undergone an endoscopy before the study period with negative findings, and are defined here as 'incident' cases. For these cases, the risk for positive findings at endoscopy within the study period was lower and dietary intake was measured closer to the onset of disease than among those who had not previously undergone endoscopy. As no important differences between incident and prevalent cases were seen in intake of calcium, vitamin D, and dairy products or in other variables of interest, both incident and prevalent cases were included in the analysis.

The occurrence of adenomatous polyps increased with age, and a history of colon or rectal cancer in either parent was positively related to the risk for developing an adenoma. Total energy intake was not associated with risk for adenoma. All subsequent analyses included age, total energy intake and family history of colorectal cancer.

Table 3.1 Characteristics[*] of the male and female study population, by the lowest and highest quintiles of calcium and vitamin D intake: The Health Professionals Follow-up Study (HPFS) and The Nurses' Health Study (NHS).

	HPFS, 1986 - 1990				NHS, 1984 - 1988				NHS, 1980 - 1988			
	calcium		vitamin D		calcium		vitamin D		calcium		vitamin D	
	Q1[†]	Q5[†]	Q1	Q5	Q1	Q5	Q1	Q5	Q1	Q5	Q1	Q5
Participants (no.)	1,897	1,898	1,898	1,898	1,454	1,454	1,455	1,456	1,787	1,784	1,785	1,787
Cases (%)	3.1	3.0	3.9	4.0	3.8	4.1	4.1	4.1	3.7	3.6	4.6	3.0
Mean age at endoscopy (years)	57.8	59.2	56.3	59.9	54.7	56.7	54.3	56.8	54.7	55.7	54.6	55.5
Mean total energy intake (kcal)	1,877	1,915	1,866	1,778	1,640	1,643	1,690	1,569	1,585	1,582	1,520	1,417
Mean intake[‡]/day of												
calcium (mg)	547	1,649	707	1,256	471	1,663	647	1,249	388	1,232	500	909
vitamin D (IU)	285	653	118	954	189	527	85	758	184	489	59	744
Mean intake[‡]/day of dietary												
fiber (g)	22	25	22	25	17	20	17	20	15	18	17	18
saturated fat (g)	24	25	26	23	22	21	22	21	29	27	29	27
protein (g)	86	96	85	94	67	77	67	77	72	86	75	80
alcohol (g)	18	10	16	12	10	6	10	7	9	5	9	7
phosphorus (mg)	1,180	1,659	1,213	1,539	929	315	955	1,261	928	1,518	1,004	1,306
Family history (%)	12	12	12	12	13	15	13	14	12	14	12	13
Previous endoscopy (%)[§]	43	47	44	47	33	36	32	37	23	25	23	26

Indication for endoscopy (%):												
blood in stool	17	15	17	15	23	20	22	20	24	21	24	21
occult blood	3	3	4	3	6	5	5	5	5	5	4	5
abdominal pain	7	7	8	7	16	14	17	14	17	16	16	15
diarrea	6	5	6	4	15	15	16	17	18	14	15	15
routine screening	65	68	63	70	43	49	45	48	40	47	44	46
Mean body mass index (kg/m²)	25	25	26	25	25	24	24	24	24	24	25	24
Current smoker (%)	9	6	9	6	27	19	30	25	27	20	28	20

*   Standardized for age at baseline.
†   Q1 = quintile 1; Q5 = quintile 5.
‡   Adjusted to total energy intake, by regression analysis.
§   The percent age of cases with previous negative endoscopies in the three study populations was 26%, 34% and 16% respectively (see text).

Among the men, screening was the indication for endoscopy in 53% of cases and 67% of the controls; among the women, 33% of cases had endoscopy for screening compared to 46% of the controls.

Bleeding, occult blood and family history were more important among male cases (21% vs. 15%; 9% vs. 3%, 11% vs. 7%, respectively) and female cases (37% vs. 22%; 7% vs. 4%, 19% vs. 13%, respectively). In multivariate models, indicator variables for previous endoscopies, indication for endoscopy, body mass index, alcohol, folate, dietary fiber and saturated fat intake were included. Physical activity was not significantly related to the risk in men or women and was not included in multivariate analyses. The mean size of polyps in both men and women was 1.0 centimeter.

Calcium intake

The median intakes of calcium in men, adjusted to 2,000 kcal/day, ranged from 560 mg/day in the lowest quintile to 1,500 mg/day in the highest. In women, based on the 1980 questionnaire, the quintiles of median total calcium intake, adjusted to 1,600 kcal/day, ranged from 400 to 1,160 mg/day, whereas the quintiles of median intake based on the extended 1984 questionnaire (which included specific questions on calcium supplements) were comparable to those of men: 480-1,550 mg/day.

The total energy-adjusted calcium intake was not associated with the risk for colorectal adenoma in men or women (relative risk (RR), highest vs. lowest quintile of intake: men, 0.91, 95% confidence interval (CI) 0.63-1.32; women, 1984-1988, 1.00, 95% CI = 0.68-1.46; women, 1980-1988, 0.94, 95% CI = 0.66-1.33). This finding persisted after potentially confounding variables in further multivariate models were controlled for (Table 3.2) and after stratification (based on tertiles) by saturated fat intake, although a modest non-significant inverse association was suggested at higher intakes of saturated fat (RR for high calcium/high fat vs. low calcium/high fat: men, 0.80, 95% CI = 0.49-1.29; women, 0.79, 95% CI = 0.49-1.25; RR for high calcium/low fat vs. low calcium/low fat; men, 1.17, 95% CI = 0.71-1.93; women, 0.95, 95% CI = 0.56-2.86).

Dietary calcium (excluding supplement use) was also unrelated to the risk for adenoma after age, total energy and family history were controlled for (RR, highest vs. lowest quintile of dietary calcium intake, men, 0.90, 95% CI = 0.63-1.28; women, 1984-1988, 1.05, 95% CI = 0.71-1.55; women, 1980-1988, 0.98, 95% CI = 0.69-1.39), and in further multivariate models (Table 3.2). Neither calcium from dairy nor from non-dairy sources was related to adenoma risk (data not shown).

The calcium/phosphorus ratio was not related to polyp risk (RR adjusting to potentially confounding variables for the highest vs. lowest quintile of this ratio: men,

1.21, 95% CI = 0.84-1.74; women, 1.01, 95% CI = 0.71-1.44), nor was there any evidence of effect modification of calcium by level of phosphorus intake.

Higher intake of calcium was non-significantly inversely related to the risk for adenoma among men in the lowest tertile of vitamin D intake (RR = 0.52, 95% CI = 0.22-1.21, for high vs. low tertile of calcium) but no such association existed in women (RR = 1.10, 95% CI = 0.55-2.17).

Table 3.2   Relative risk[*] of colorectal adenoma among men and women, by quintiles of calcium intake and vitamin D intake: The Health Professionals Follow-up Study (HPFS) and The Nurses' Health Study (NHS).

| | Study population | | | | | |
| | HPFS, 1986-1990 | | NHS, 1984-1988 | | NHS, 1980-1988 | |
Calcium intake[‡]	Total	Dietary	Total	Dietary	Total	Dietary
Quintile						
1 (ref)	1.00	1.00	1.00	1.00	1.00	1.00
2	1.36	0.98	0.91	1.19	1.04	1.07
3	1.41	1.10	1.29	1.01	1.49	1.50
4	1.33	1.21	1.15	1.59	1.13	1.11
5	1.13	1.01	1.16	1.18	1.17	1.21
95% CI[†] Q5 vs. Q1[†]	0.76-1.66	0.70-1.46	0.78-1.72	0.79-1.78	0.81-1.69	0.84-1.74
Trend p[§]	0.98	0.77	0.37	0.27	0.47	0.42
Vitamin D intake[‡]	Total	Dietary	Total	Dietary	Total	Dietary
Quintile						
1 (ref)	1.00	1.00	1.00	1.00	1.00	1.00
2	0.96	0.90	0.89	0.92	1.09	0.86
3	0.83	1.00	0.91	0.80	0.80	1.07
4	0.68	0.78	0.82	0.80	0.88	0.90
5	1.29	1.05	1.04	1.09	0.68	0.97
95% CI Q5 vs. Q1	0.87-1.93	0.74-1.49	0.65-1.67	0.75-1.58	0.41-1.13	0.68-1.38
Trend p[§]	0.32	0.82	0.86	0.64	0.09	0.93

[*]   Adjusted for age, total energy intake, body mass index, alcohol consumption, folate, saturated fat and fiber intake, indications for endoscopy, history of endoscopy prior to the study period, and family history in logistic regression analysis.
[†]   CI = 95% confidence interval.
[‡]   Energy-adjusted by regression analysis.
[§]   Test for trend calculated by using the median of each quintile of intake as a continuous variable in logistic regression.

Table 3.3   Relative risk[*] of left colon and rectal adenoma among men and women, by quintiles of energy-adjusted[†] calcium and vitamin D intake: The Health Professionals Follow-up Study and The Nurses' Health Study (NHS).

Nutrient	Study population			
	HPFS, 1986-1990		NHS, 1980-1988	
Total calcium intake	Left colon (n=251)	Rectum (n=80)	Left colon (n=224)	Rectum (n=126)
Quintile 1 (ref)	1.00	1.00	1.00	1.00
2	1.25	1.93	0.91	1.26
3	1.15	2.59	1.52	1.40
4	1.22	1.85	1.17	1.07
5	1.02	1.61	1.33	0.90
95% CI Q5 versus Q1[‡]	0.66-1.58	0.68-3.82	0.85-2.08	0.48-1.71
Trend p[§]	0.86	0.78	0.15	0.50
Total vitamin D intake	Left colon	Rectum	Left colon	Rectum
Quintile 1 (ref)	1.00	1.00	1.00	1.00
2	0.96	0.95	1.26	0.86
3	0.83	0.86	0.91	0.67
4	0.72	0.58	1.15	0.54
5	1.35	1.00	1.05	0.30
95% CI Q5 versus Q1	0.86-2.13	0.44-2.26	0.56-1.93	0.13-0.71
Trend p[§]	0.26	0.82	0.98	0.005

[*]   Adjusted for age, total energy intake, body mass index, alcohol consumption, folate, saturated fat and fiber intake, indications for endoscopy, history of endoscopy prior to the study period, and family history in logistic regression analysis.
[†]   Adjusted by regression analysis.
[‡]   CI, confidence interval.
[§]   Test for trend calculated by using the median of each quintile of intake as a continuous variable in logistic regression.

Calcium intake was not associated with the risk for polyps of the left colon or the rectum (Table 3.3) and no differences were observed between small and larger polyps (Table 3.4). Calcium intake was not associated with risk for either prevalent or incident cases in men (prevalent cases, RR = 1.11, 95% CI = 0.71-1.73; incident cases, RR = 1.30, 95% CI = 0.57-2.94) and women (data not shown).

Vitamin D
The median total energy-adjusted vitamin D intake, in men ranged from 125 to 851 IU/day between the first and fifth quintiles, vitamin D intake from dietary sources

ranged from 101 to 470 IU/day. In women, the median total energy-adjusted intake ranged from 61 to 653 IU/day in 1980 and from 87 to 682 IU/day in 1984; dietary vitamin D ranged from 52 to 315 IU/day in 1980, and from 72 to 329 IU/day in 1984.

Table 3.4   Relative risk[*] of small (< 1 cm) and large (≥ 1 cm) adenoma among men and women, by quintiles of energy-adjusted[†] calcium and vitamin D intake: The Health Professionals Follow-up Study (HPFS) and The Nurses' Health Study (NHS).

Nutrient		Study population			
		HPFS, 1986-1990		NHS, 1980-1988	
Total calcium intake		Small (n=151)	Large (n=128)	Small (n=173)	Large (n=145)
Quintile	1 (ref)	1.00	1.00	1.00	1.00
	2	1.11	1.73	0.91	1.16
	3	1.18	1.82	1.71	1.14
	4	1.13	1.40	1.24	1.13
	5	1.22	1.16	1.05	1.26
95% CI Q5 versus Q1[‡]		0.72-2.09	0.61-2.22	0.62-1.79	0.72-2.19
Trend p[§]		0.51	0.72	0.75	0.49
Total vitamin D intake		Small	Large	Small	Large
Quintile	1 (ref)	1.00	1.00	1.00	1.00
	2	0.77	1.47	1.22	1.04
	3	0.83	1.01	1.04	0.68
	4	0.57	0.89	0.87	1.02
	5	1.05	1.67	0.65	0.85
95% CI Q5 versus Q1		0.59-1.89	0.87-3.22	0.31-1.34	0.39-1.83
Trend p[§]		0.94	0.36	0.15	0.71

[*]   Adjusted for age, total energy intake, body mass index, alcohol consumption, folate, saturated fat and fiber intake, indications for endoscopy, history of endoscopy prior to the study period, and family history in logistic regression analysis.
[†]   Adjusted by regression analysis.
[‡]   CI = confidence interval.
[§]   Test for trend calculated by using the median of each quintile of intake as a continuous variable in logistic regression.

When age, energy intake and family history were adjusted for, a slight inverse, non-significant association for energy-adjusted vitamin D intake was observed in men (RR = 0.85, 95% CI = 0.61-1.19). In multivariate analysis, this association disappeared (Table 3.2). In the 1984-1988 follow-up among women, a similar lack of association was observed (Table 3.2). In the 1980-1988 follow-up among women, total vitamin D

intake was inversely associated with the risk of adenoma (RR = 0.61, 95% CI = 0.43-0.87, p = 0.002), when age, total energy intake and family history were adjusted for. This association did not remain significant in multivariate analysis (Table 3.2), which included folate intake. The Pearson correlation between folate and vitamin D intake in this cohort was 0.58.[52] No clear trend with duration of multivitamin use was evident.

The inverse association with total vitamin D intake among women in the 1980-1988 follow-up was significant for rectal adenomas (Table 3.3), for which an inverse association with dietary vitamin D intake was observed as well (RR highest vs. lowest quintile = 0.64, 95% CI = 0.35-1.17). No important differences were observed between cases with large polyps versus those with small ones (Table 3.4) or between prevalent and incident cases in men (prevalent cases, RR = 1.09, 95% CI = 0.68-1.76; incident cases, RR = 2.07, 95% CI = 0.96-4.46) or women in the 1984-1988 follow-up (data not shown). The apparent inverse association with vitamin D in the 1980-1988 follow-up was only observed in women who had not previously undergone endoscopy (prevalent cases, RR = 0.53, 95% CI = 0.30-0.93; incident cases, RR = 2.19, 95% CI = 0.64-7.53). However, in this follow-up period, the number of incident cases (16%) was too small for drawing firm conclusions.

Dairy products
After 4 years of follow-up, no association was found between the consumption of whole milk or low-fat or skim milk and the risk for colorectal adenoma in men or women (Table 3.5). In the 1980-1988 follow-up, low-fat or skim milk was inversely, although not significantly, related to the risk for adenoma (RR = 0.87, Table 3.5).

The fermented dairy products (yogurt, sour cream, cottage cheese, cream cheese and other cheese) combined either by calcium content (data not shown) or by the weight of the normal portion size were not associated with the risk for adenoma in men (Table 3.5). For women in the 1984-1988 and 1980-1988 follow-ups, a nonsignificant inverse association was observed. Adjusting for saturated fat intake did not change these findings appreciably.

For specific fermented dairy products an inverse nonsignificant association was observed for yogurt in men and women (RR for consumption of 1 cup more than four times per week vs. almost never: men, 0.66; 95% CI = 0.28-1.46, trend p = 0.07; women, 1984-1988, 0.85, 95% CI = 0.43-1.70, trend p = 0.10; women, 1980-1988, 0.60, 95% CI = 0.30-1.18 trend p = 0.11).
Among men, this association became weaker when saturated fat intake was adjusted for (RR for consumption of 1 cup more than four times per week vs. almost never = 0.70, 95% CI = 0.31-1.62, trend p = 0.19) and it further diminished controlling

for other potential confounders (RR for consumption of 1 cup more than 4 times per week vs. almost never = 0.89, 95% CI = 0.39-2.07, trend p = 0.63). Among men an inverse association with sour cream consumption was observed (RR for consumption of 1 tablespoon more than once per week vs. almost never = 0.67, 95% CI = 0.37-1.22), and this remained after adjustment for saturated fat intake (RR = 0.61, 95% CI = 0.33-1.11, trend p = 0.06) and other potential confounders (RR for consumption more than once a week vs. never = 0.62, 95% CI = 0.34-1.13, trend p = 0.07).

Sour cream consumption was not associated with risk in women in the 1984-1988 follow-up (RR for consumption of 1 tablespoon more than once per week vs. never = 0.88, 95% CI = 0.48-1.61, trend p = 0.54). In 1980 the questionnaire did not include this product. Hard cheese, a fermented product with a high fat content was positively associated with the risk of colorectal polyps in men (RR for consumption of 1 slice or 1 ounce serving more then 4 times a week vs. never = 1.45 (Table 3.5). After adjustment for saturated fat intake, this association diminished (RR = 1.28, Table 3.5). Adjusting for other possible confounders did not materially affect this. The positive association with hard cheese was not observed for women in the 1984-1988 follow-up (RR = 1.16, Table 3.5), nor those in the 1980-1988 follow-up (RR = 0.91, Table 3.5). The consumption of cottage cheese and cream cheese was not related to adenoma risk in men and women (data not shown).

Discussion

These prospective data do not support an important association of calcium or milk intake with the occurrence of colorectal adenomas. Some slight support for the hypothesis that vitamin D intake is protective against rectal adenoma is provided for women using the 1980 questionnaire, but no effect of vitamin D on adenoma risk was observed among men and in the 1984-1988 follow-up among women.

Most studies on calcium or milk intake and colorectal cancer development examined the ultimate disease and not colorectal adenomas. If calcium indeed influences the cell proliferation of colonic epithelium,[5,6] the failure to find an inverse association with cancer could be due to a long time period required for the development of cancer. Our analyses do not suggest an effect of calcium on adenoma development or progression, as no differences between small and larger adenomas were observed. Our findings on calcium and milk intake are consistent with a hospital-based case-control study[37] on adenomas (RR for calcium, highest versus lowest quartile = 1.28; RR for milk, highest versus lowest quartile = 0.90).[37]

Moreover, in a screening-based case-control study of colorectal adenomas, no association was observed (or was not presented).[39]

Previous epidemiologic studies focusing on colorectal cancer and milk consumption or calcium intake have not been conclusive. A population-based case-control study in Utah suggests a significant inverse association between calcium and dairy products and colorectal cancer risk: men and women in the upper quartile of intake had half the risk of those in the lowest quartile.[11] A non-significant inverse association between milk consumption and risk of colon cancer was observed among Seventh Day Adventists in the United States,[15] while other case-control studies do not support an inverse association with calcium and/or milk consumption.[16-18]

In a prospective study on colorectal cancer among 11,888 residents of a retirement community in California, that used a self-administered food frequency questionnaire listing 56 foods or food groups 126 incident cases were confirmed after 4 years of follow-up.[14] In this study, a slight, but not significant association with calcium intake was observed (RR for men, highest vs. lowest tertile = 0.86, 95% CI = 0.4-1.7).

One prospective study, using 28-day dietary histories suggested a significant inverse association between calcium and the risk for colorectal cancer (49 cases) among 1,954 male employees of the Western Electric Company.[13] Based on the results of this study and the Utah case-control study, Garland and co-workers suggest a linear dose-response relation between the intake of 700-1,200 mg of calcium per day and colon cancer risk. They hypothesize a very low incidence for calcium intake of 1,800 mg/day in men and 1,500 mg/day in women.[53] According to the calculations of Newmark et al.[5], a calcium intake of 1,500 mg/day or more is necessary for individuals with diets high in fat (150 g/day), and phosphate (1,500 mg/day), to neutralize the average fatty acid and bile-acid concentration in the bowel. In our studies, 11% of the male population had an intake of 1,500 mg/day or more, and 4% of the female population had a daily intake of 1,500 mg/day or more in 1980, compared to 15% in 1984 (calcium-containing supplements were not included on the 1980 questionnaire). A substantial proportion of participants in our studies should have been consuming an adequate amount of calcium to experience the biologic effects, especially since fat intake was lower than used in the calculations of Newmark et al. (mean, 69 g/day in women and 80 g/day in men).[47,50]

The failure to demonstrate an association with calcium intake may be explained by the relatively low intake of saturated fat and high intake of dietary fiber among men in the HPFS compared to the general male population.[39] However, the association with calcium did not differ at different levels of saturated fat or dietary fiber intake. Neither was the calcium/phosphorus ratio associated with adenoma risk.

In a cohort of Hawaiian-Japanese men, total calcium intake, estimated using 24-hour recalls, was not related to colorectal cancer on the whole,[54-56] but an inverse association

was observed for sigmoid colon cancer.[56] We did not observe a difference of the effect of calcium between subsites among men or women. Alternatively, the proliferative effect of fatty acids and bile acids may be especially important for the right side of the colon.[57] If calcium binds these substances and thereby reduces the risk of colon cancer, this protective effect may be most important for the caecum and ascending colon. An effect on right-sided polyp risk could not be assessed in our study.

A possible protective effect of vitamin D was initially suggested by the data for the women in the 1980-1988 follow-up, but could not be confirmed during the 1984-88 follow-up of the women or the 4-year follow-up of the men. Latency time did not explain this difference: when the 8-year follow-up using the 1980 questionnaire was separated into two 4-year periods, the inverse association with total vitamin D intake remained in both periods. The apparent protective effect among women during the 1980-1988 follow-up seemed to be mainly attributable to multivitamin supplements containing vitamin D. The 1980 version of the questionnaire included questions on multivitamin use (dose, frequency, brand name, and duration), but no separate questions on the intake of calcium or vitamin D supplements. However, the use of multivitamins contributed substantially to vitamin D intake. The observed association with total vitamin D intake may be difficult to interpret because of the intercorrelations with other vitamins and minerals in multivitamin supplements that include folate.[52] As diet is probably measured closer to the onset of disease in women who did undergo endoscopy before the study period, a stronger association with vitamin D intake would have been expected. This was not confirmed by our findings; the association with vitamin D intake was stronger among 'prevalent' cases. (Note: this study addresses dietary and supplemental intake of vitamin D, and does not take sunlight exposure into account).

In the Western Electric cohort, a dietary intake of more than 3.75 μg (150 IU/day) of vitamin D per day was associated with a 50% reduction in the incidence of colorectal cancer in men.[13] In a prospective nested case-control study of 8,006 Hawaiian-Japanese men (159 cases) using a 24-hour recall method, a slight negative association with rectal cancer risk was observed.[55]

The intake of fermented dairy products as a single food group did not seem to be related to the risk for colorectal adenomas. The median of intake in the highest quintile was 111 g/day for men and 128 g/day for women using the 1986 and 1984 questionnaire, respectively. This contrasts with intakes of 225 g/day or more in a Dutch case-control study in which fermented dairy products were seen to have a protective effect against breast cancer.[28] Thus, the limited range of intake in our population may have reduced the power to detect the hypothesized association with fermented products. Combining fermented products into one food group has disadvantages, as the products

differ in their fat content, strains of microorganisms and enzymes. Differen microorganisms might have different effects on the bowel flora or immunocompeten cells in the colon mucosa.[33] Analyses using single food items were limited by the large number of participants who reported infrequent consumption of particular products and the restricted range of intake.

In summary, these data do not support an inverse association between calcium intake and the occurrence of adenomatous polyps in men and women. The slight inverse association with vitamin D observed in only one of the two data sets of women was mainly attributable to the use of vitamin supplements that include other potentially protective components such as folate,[52] and should therefore be interpreted with considerable caution. However, the observation of a stronger association of recta polyps with vitamin D from both supplements and diet in women suggests that further investigation may be useful. These data generally do not support the existence of othe factors in dairy products protective against colorectal adenoma. However, they do no exclude an effect of higher levels of intake of dairy products, nor do they exclude an effect in the proximal colon. In addition, further study is required to examine the potential influences of dairy products on later stages of carcinogenesis.

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Chapter 4

Design, conduct and validity of the case-control study

Contents

Extended abstract

Case recruitment
Control recruitment
Non-response
Collection and validity of dietary intake data
Implications

Study population

Principles

Definition of cases and controls
Study size and duration

Recruitment of cases

Procedures of case recruitment
Cooperation of hospitals
Eligibility of invited cases
Response and participation of cases
Determinants of invitation and participation
Implications of case recruitment

Recruitment of controls

Rationale
Procedures for control recruitment
Cooperation of general practitioners
Cooperation of municipal registries
Participation of GP-mediated controls
Municipal and GP-mediated controls
Implications of control recruitment

Indicators of non-response

Collection and validity of the data

Short questionnaire
Home interview
Alterations of the questionnaire during the interview period

Extended abstract

Since selection bias is an inherent concern in case-control studies, this chapter concentrates on recruitment of cases and controls, and discusses its implications regarding internal and external validity. The different steps in the recruitment procedures are evaluated, which might be of special interest to those intending to conduct a case-control study on cancer in the Netherlands. Furthermore, the collection and validity of the dietary data are discussed and some issues relevant to potential information bias are evaluated.

Case recruitment

Recruitment of cases was planned to cover two and a half years, in which dietary history data of 250 cases (ICD-O 153) and 250 age- and sex- stratified controls would be gathered in the catchment area of the Comprehensive Cancer Centre Middle Netherlands. Cases were invited by surgeons in general hospitals covering the area. The cancer registry was used as a check of completeness and provided additional clinical and pathological information. In the first year of study conduct, two major hospitals in the study area appeared to be non-cooperative and invitation and participation rates were less than expected. Therefore, recruitment of cases was extended by half a year to a period of three years and two additional hospitals in the catchment area of the Comprehensive Cancer Centre Eastern Netherlands were included.

To check whether invitation and participation of colon cancer cases were subject to selection, the database of the cancer registries was linked to the study database. As complete registration by the cancer registries takes place 3 to 6 months after diagnosis, linkage was conducted three months after the last case was interviewed. Surprisingly, it appeared that about one third of the 478 invited cases did not fulfil the eligibility criteria. Main reasons for ineligibility were non-colon tumors (rectosigmoid, rectum) and secondary primaries.

Of the eligible subjects who got colon cancer during the study period, and who were treated in cooperating hospitals, 47% were invited by their surgeon; more than 50% of these were invited after the surgeon had been notified by the cancer registry. Of those invited, 72% replied, 69% filled out a short questionnaire and 60% were additionally willing to participate in the home interview. The 200 eligible participants differed significantly from the total of eligible patients only according to tumor status: subjects with Dukes' A and B tumors were overrepresented, since these less severe cases were invited more frequently by their surgeons.

Control recruitment

To increase response of controls, efficiency of procedures and to match cases to their source population, controls were recruited using the database of the general practitioner (GP) of the case interviewed. Of the GP-mediated controls invited, 79% replied, 75% filled out the short questionnaire, and 57% were willing to be interviewed. The 259 eligible participants did not differ with respect to age and gender from those invited, which resembled the distribution among the cases. Participants lived more frequently in urban areas and were more often privately insured than the non-participants. The proportion of privately insured (38%) closely resembled the proportion in the general population in 1992 (32%) according to the Dutch Central Bureau of Statistics.

   To study whether the GP-mediated procedure of control recruitment did indeed increase response, but introduced some biases, demographic characteristics and dietary habits of GP controls living in urban areas (n = 249) were compared with an age-stratified sample (n = 301) drawn from municipal registries of four representative towns. Response of the urban GP-mediated controls was higher than that of the municipal controls (78% vs. 71%). GP-mediated and municipal controls did not differ in frequency of visiting their GP. The urban controls recruited by these procedures differed in proportion of privately insured (GP-mediated 36%, municipality-mediated 57%), in the proportion of men (54% vs. 44%) and in intake of fermented dairy products (in highest category of intake 29% vs. 37%).

Non-response

With a short questionnaire briefly inquiring about dietary patterns, it was planned to gain information on habits of non-responders, i.e. those who did not participate in the interview. However, only 23% of the cases not interviewed and 42% of the non-participating controls completed the questionnaire. Non-participating cases who filled out the short questionnaire were more frequently female and consumed more fermented dairy products than interviewed cases. Non-participating controls who completed the short questionnaire did not differ in demographic or medical factors nor in dietary patterns. Unfortunately, firm conclusions cannot be drawn from these observations since those non-participants who completed the questionnaire may not be representative of the total of non-responders.

Collection and validity of dietary intake data

The structured dietary history questionnaire was based on the validated questionnaire used in a previous case-control study on breast cancer. The part assessing the consumption of dairy products was extended and additional cross-checks on the internal consistency of the frequency of various meal components

with the total meal pattern were implemented.

Interviewing was conducted by seven dieticians who interviewed a similar number of cases and controls. No important differences were observed between interviewers.

Each retrospective study may be prone to recall bias, which might differ between cases and controls. As it is expected that cases may generally overestimate their dietary intake, it is very important to adjust nutrient intakes for total energy intake in the data analyses. Since the participants were unaware of the hypothesis tested and dairy products have a relatively positive health-image, it is unlikely that cases have systematically over- or underestimated their dairy product consumption.

Implications

The foregoing might have implications for internal and external validity.

Internal validity implies validity of inference for the study subjects themselves. A loss of power of 6% is due to the fact that about 30% of the orginally interviewed cases turned out not to be eligible for the study. Still the power to detect an odds ratio (OR) of 0.5, assuming $\alpha = 0.05$ two-sided and $\beta = 0.1$, will be ca. 80% when 200 cases and 259 controls are included in the analyses. For reasons of efficiency, cases who were 75 years old at diagnosis and 76 years old when interviewed will be included in the study.

Moreover, since the definition of rectosigmoid might differ between clinicians and the cancer registries, analyses may be conducted including as well as excluding the 28 assumed rectosigmoid cases.

The fact that participating cases have a better prognosis than non-participants may have a positive effect on the validity of the dietary data. No association between tumor status and the consumption of fermented dairy products was observed, which implies that the inclusion of cases with a relatively better prognosis in the study is not likely to influence the results importantly.

The consumption of fermented dairy products might differ between participating and non-participating cases and between participating and non-participating controls in a different way. Although the data must be interpreted with considerable caution due to the small group of non-participating cases who filled out the short non-response questionnaire, the consumption of fermented dairy products may be lower for the participating cases than for the total case group. Although the difference was not significant, participating controls consumed fermented dairy products more frequently than those only filling out the non-response questionnaire. The possible selection of cases and controls will strenghten the hypothesized inverse association. As a consequence, when a significant inverse association will be observed, one
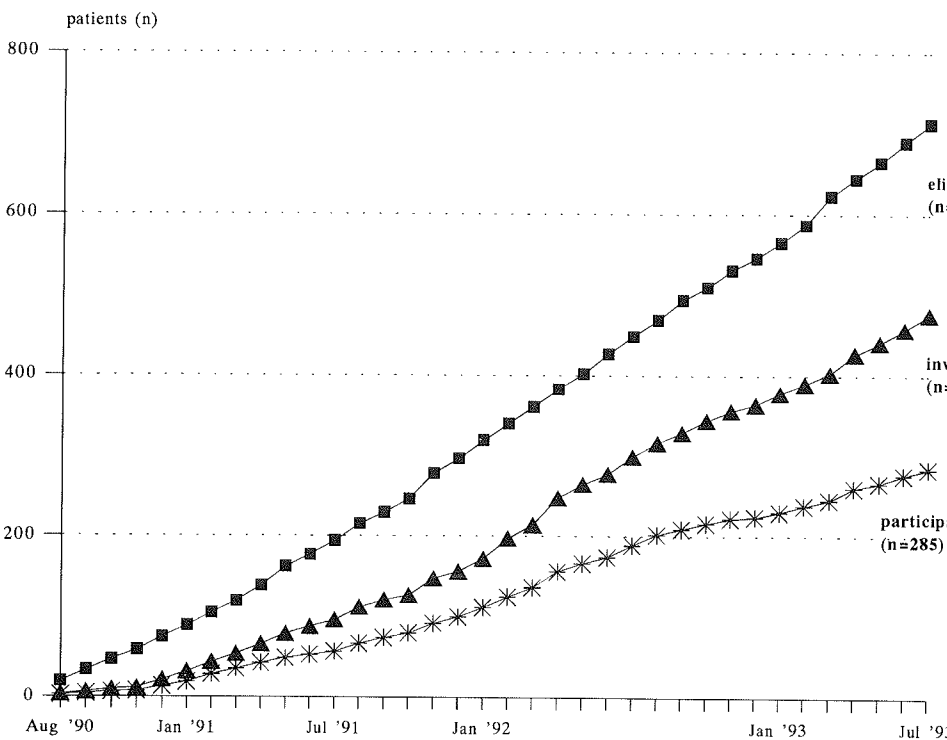
Figure 4.1   Recruitment of cases in time.

Recruitment of cases

Procedures of case recruitment

To be able to approach as many eligible cases as possible, two complementary procedures of case recruitment were applied (Figure 4.2).

According to the first procedure, surgeons identified patients meeting the eligibility criteria, and gave them a letter of invitation during their stay in the hospital, shortly after diagnosis. All cases seen by the surgeon were notified to the researchers demographic characteristics (gender, date of birth and place of residence) were reported. If the patient was not invited for medical or psychological reasons, or because the patient did not match the inclusion criteria, the reason for non-invitation was recorded.

   The second recruitment procedure was meant to identify cases possibly missed by the first procedure because they were not invited by the surgeon. Demographic data from the list of cases notified to the researchers were linked to the database of the cancer registries involved. If an eligible case appeared not to have been invited in the first procedure, the cancer registries notified the surgeons and asked to mail the letter of invitation to the patient's home address or to invite the patient during the

Procedure 1   **Procedure 2**
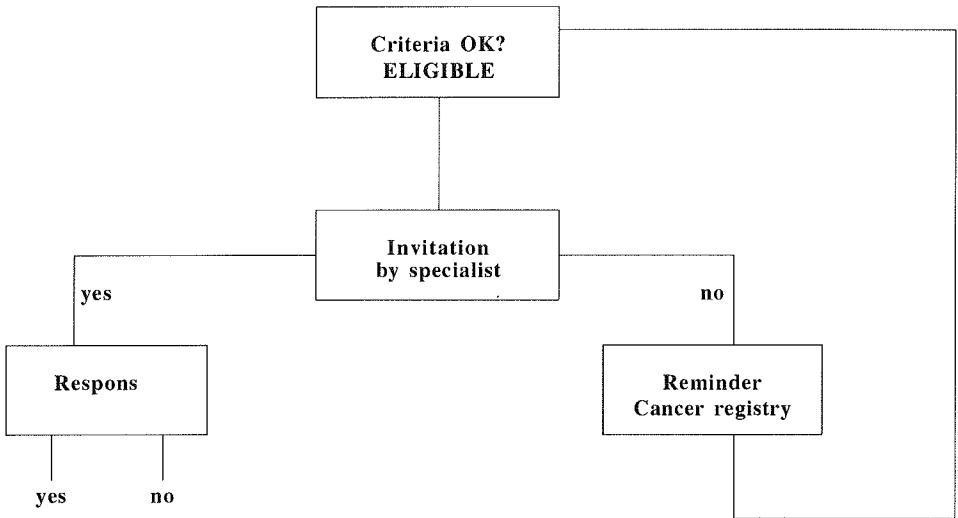
Figure 4.2   Recruitment of cases by two complementary procedures.

The cooperation of the cancer registry in case recruitment appeared to be crucial as 50% of those invited and 49% of the eligible interviewed cases were recruited through this procedure (Figure 4.3).

	total invited	invited eligibles	participants
directly invited	239	152	103
via CR	239	180	97

Figure 4.3   Invitation and participation of cases according to recruitment procedure: by direct invitation (procedure 1) or via Cancer Registry (procedure 2).

Cooperation of hospitals

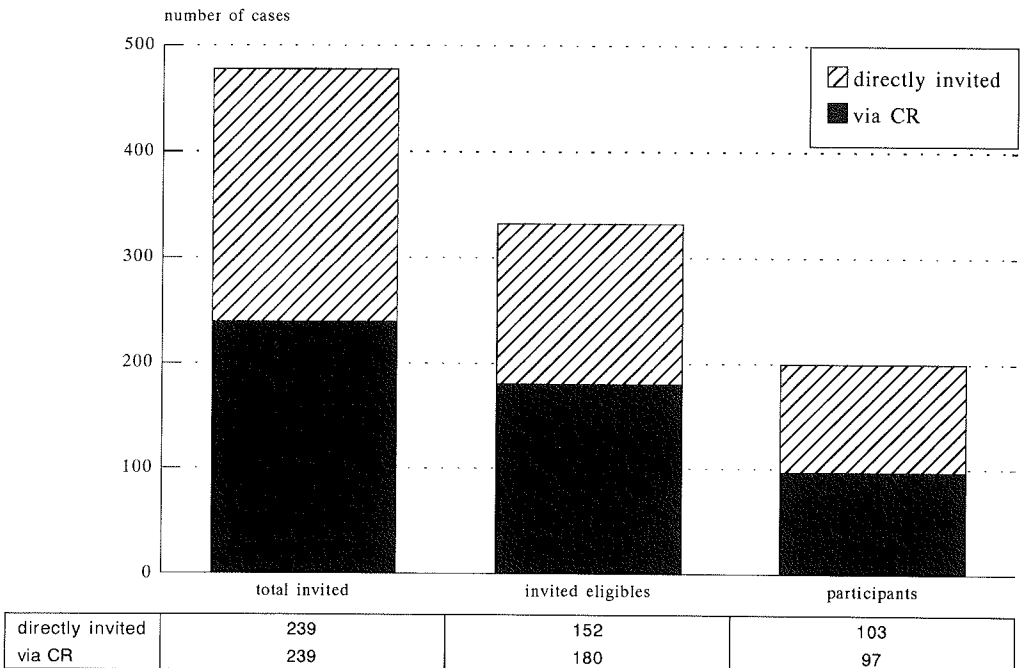Recruitment procedures appeared to differ among hospitals. In some hospitals, the majority of the cases were asked to participate directly after surgery when they were still in the hospital, without interference of the cancer registry, while other hospitals relied mainly on the cancer registry procedure. Besides these differences in procedures used, the actual rate of invitation differed among hospitals. In Figure 4.4. the cooperating hospitals are ordered according to number invited as a proportion of those eligible. The invitation rates in the different hospitals varied between 78% and 6%. Figure 4.4 shows that participation rates of cases varied among hospitals as well, which might be explained by the different personal approaches used by the surgeons and the different recruitment procedures used. Of the eligible cases who were invited directly by the surgeon 68% (103/152) participated, while the participation rate was 54% (97/180) for the cases invited after interference of the cancer registry (Figure 4.3).

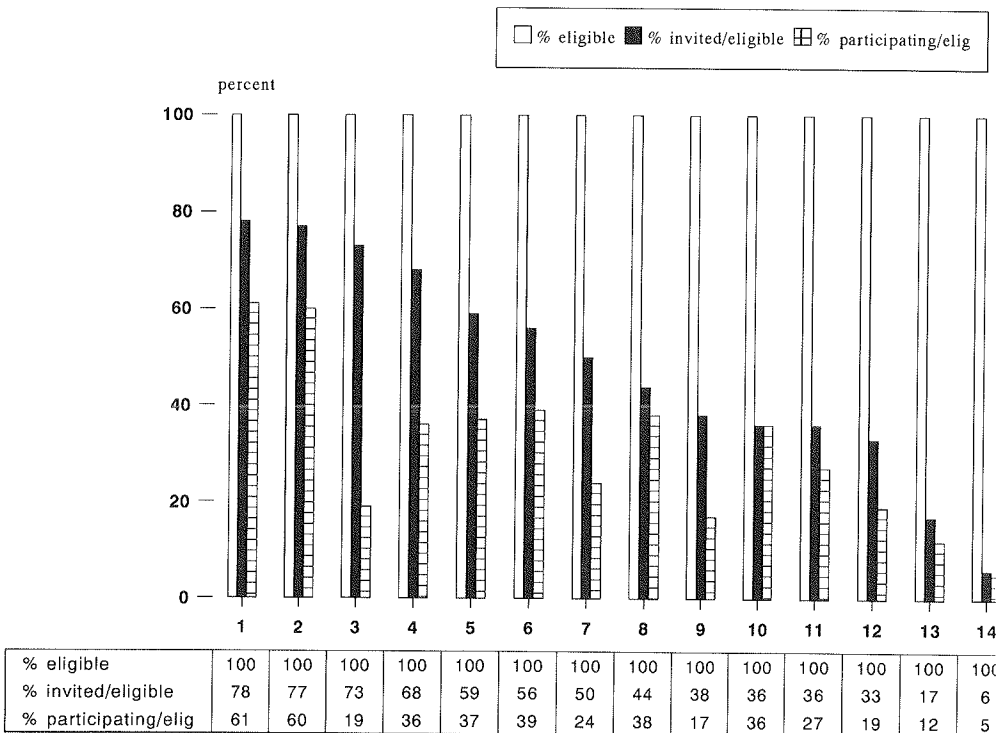|	1	2	3	4	5	6	7	8	9	10	11	12	13	14
% eligible	100	100	100	100	100	100	100	100	100	100	100	100	100	100
% invited/eligible	78	77	73	68	59	56	50	44	38	36	36	33	17	6
% participating/elig	61	60	19	36	37	39	24	38	17	36	27	19	12	5

Figure 4.4   Recruitment per hospital.

Eligibility of invited cases

Figure 4.5 presents the relation between the study database and the cancer registry

data, for different stages in recruitment. From August 1990 to August 1993, 2064 colon cancer cases were recorded by the Comprehensive Cancer Centres Middle and Eastern Netherlands (Figure 4.5), of which 1412 were seen in cooperating hospitals. Of these 1412 colon cancer patients, 677 (48%) met the inclusion criteria known by the cancer registries (adenocarcinoma, 75 years or younger, no previous tumors), and did not decease before they could be invited. Of these 677 eligible cases, 445 (66%) were reported to the research team during the study. During the recruitment period, surgeons notified another 47 cases to the research team of which 36 seemed to be eligible; these cases however did not appear in the cancer registry database. This might be mainly due to the fact that comparison with the cancer registry database took place three months after the last interview, while final registration lasts until six months after surgery.

These facts explain why the number of participants (Figure 4.1) exceeds the number of interviewed eligible cases (n = 200) in Figure 4.5.

During three years of recruitment, 478 patients were invited (Figure 4.1 and 4.3). Unexpectedly, for the researchers as well as the cancer registries, 146 (31%) of these cases did not meet the eligibility criteria. Most of these ineligibles were identified through linkage to the cancer registries (87%), whereas 19 cases (13%) were found to be ineligible during the home interview. The main reasons for ineligibility were the site of the tumor (according to the cancer registries: 43 rectosigmoid, 41 rectum, 3 other sites outside the large bowel) and the fact that 36 colon tumors were secondary primaries. Further ineligibility resulted from the malignancy grade or morphology of the tumor (7 tumors in situ, 4 metastasis of other primaries, 2 non-adenomatous) or was related to non-tumor related criteria (7 cases older than 75, 1 did not speak Dutch, 2 were not western Europeans and 5 deceased before the interview could take place). As final registration of cases by the cancer registry, including the more precise localization of the tumor, takes place six months after surgery, these types of errors are difficult to prevent. After all, to diminish recall bias and the number of deceased subjects, cases should have been interviewed as soon as possible after diagnosis, which was before final registration could take place.

It should be realized that the fact that ineligible cases were invited implies that ineligible cases participated in the interview (n = 85), and the decision to exclude them from data analysis would result in a loss of statistical power.

Response and participation of cases

All invited cases were asked to return a reply form on which they could indicate their (un)willingness to participate in the detailed interview at home (see 'Collection

about dietary patterns. Non-participants and participants in the home interview were
asked to complete the questionnaire to be able to define the non-response (see
'indicators of non-response').

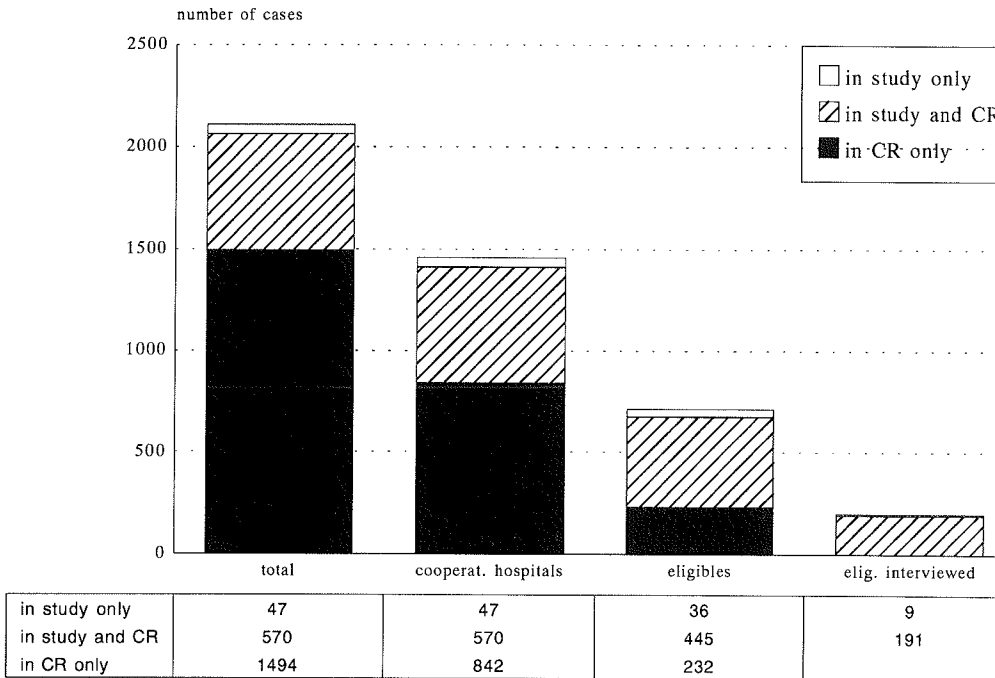|	total	cooperat. hospitals	eligibles	elig. interviewed
in study only	47	47	36	9
in study and CR	570	570	445	191
in CR only	1494	842	232	

Figure 4.5   Number of colon cancer cases in the study and in the databases of the cancer registries
(CR).

If an invited case replied to be willing to participate, the home interview took
place three to six months after diagnosis. Of the 322 invited eligibles, 240 (72%)
returned the reply form, 230 (69%) completed the short questionnaire, while 200
(60%) participated in the interview (Table 4.2).

Determinants of invitation and participation
With the exception of cases under 40 who were invited least frequently, numbers of
eligible colon cancer cases invited were inversely related to age (Table 4.2, first
column). Patients with Dukes' A or B tumors and those who underwent only an
operation were more likely to be invited than patients with Dukes' C or D tumors
or those who underwent a therapy other than an operation or had additional therapy
(e.g. radiation, chemotherapy). Invitation was not related to gender, degree of
urbanization (urban or rural) and tumor site (left- or right- sided) (Table 4.2).

Table 4.2 Response rates, stratified according to demographic variables, of eligible colon cancer cases (n = 713).*

	Invited[1] (%) (n=332)	Replied[2] (%) (n=240)	Filled out short questionnaire[3] (%) (n=230)	Participated in interview[4] (%) (n=200)
Overall response	47	72	96	87
Demographic variables				
age < 40 years	39	74	100	82
40-49	54	82	100	86
50-59	49	77	98	92
60-69	48	71	94	86
69-75	43	66	93	87
gender male	49	72	96	93
female	44	73	96	81*
regionurban	88	69	97	86
rural	85	80	96	89
unknown$	2	20	0	0
Medical factors				
tumor status				
Dukes' A and B	58	76	96	89
Dukes' C and D	45*	73	95	87
unknown$	31	60	97	81
therapy (%)				
no therapy	0	0	0	0
operation	54	72	95	87
other (additional) therapies	43*	80	100	91
unknown$	32	67	97	82
tumor site (%)				
right-sided	50	70	94	91
left-sided	47	75	97	84
unknown$	35	67	100	85

[1] invited as a percentage of eligible.
[2] replied as a percentage of invited.
[3] short questionnaire as a percentage of replied.
[4] interview as a percentage of short questionnaire.
$ the category 'unknown' was not included in analyses of heterogeneity.
* heterogeneity in response rate ($p < 0.05$).

Additionally, Table 4.2 presents the response rates by demographic and medical characteristics for those eligibles who returned the reply form (second column), those who completed the short questionnaire (third column) and those who eventually participated in the interview at home (fourth column).

Response rates were somewhat higher among younger persons (except for those under 40 years) and among those living in rural areas.
Although response rates and the willingness to fill out the short 'non-response' questionnaire did not differ between genders, men were more inclined to participate in the interview.

Table 4.3   Distribution of demographic and medical characteristics of total eligible colon cancer cases and those eligible cases participating in the home interview.

Variables	Colon cancer cases	
	Total eligible (n = 713)	Participants in home interview (n = 200)
Demographic variables		
age (%) < 40 years	8	7
40-49	9	12
50-59	19	22
60-69	36	36
69-76	29	24
gender (% male)	51	57
region (%)[1]		
urban	64	60
rural	36	40
Medical factors		
tumor status (%)[2]		
Dukes' A and B	57	64
Dukes' C and D	43	36*
therapy (%)[3]		
no therapy	2	0
operation only	80	82
other (additional) therapies	19	18
tumor site (%)[4]		
right-sided	47	49
left-sided	53	51

*   differs from non-participants (53%; p < 0.05), the category 'unknown' is not included in the analyses of heterogeneity.
[1]   unknown of total eligible: 47%.
[2]   unknown 28% and 16% respectively.
[3]   unknown 23% and 14% respectively.
[4]   unknown 12% and 9% respectively.

Tumor status, therapies and tumor site were not appreciably related to response rates. Of course, one should realize that this may simply reflect the fact that the surgeons had already limited their invitations to subjects who were in fair condition

and able to participate.

For interpreting the relevance of the separate stages in selection identified above, the initial and final distribution of demographic and medical characteristics is of interest. The more frequent participation of less severe cases being the only exception, the distribution of demographic and medical characteristics of the final eligible cases participating in the interview did not differ markedly from the initial eligibles (Table 4.3).

This implies that, relatively, more healthy cases participated in the interview. However, since illness, radiotherapy and chemotherapy might influence dietary patterns and thus might hamper the recall of habitual diet, excluding cases with a worse prognosis is likely to favour the quality of the interview data.

Of course, the crucial issue for inference from the case-control study is whether the distribution of the exposures of interest, fermented milk consumption and calcium intake, is altered by the subsequent selection steps. Although this cannot be studied throughout all subsequent steps, we intended to obtain limited dietary data from all cases using a short questionnaire. A similar attempt was made among non-participating controls. Results of both groups are described in 'Indicators of non-response'.

Implications of case recruitment

Invitation and participation rates estimated in the planning of the study differed from those actually observed; invitation and participation were both estimated to be 80% and turned out to be 47% and 60% respectively. Therefore the period in which case recruitment took place had to be extended from 2½ to 3 years and two hospitals in the east part of the Netherlands were included.

Surprisingly, about one third of the interviewed cases did not meet the inclusion criteria of the study. Instead of the planned power of 86%, an odds ratio of 50% can be detected with a power of about 80% when 200 cases and 250 controls are included in the study. For reasons of efficiency, cases who were 75 years old at diagnosis and 76 years old when interviewed, were included in the study. Moreover, since the definition of rectosigmoid might differ between clinicians and the cancer registries,[4] analyses were conducted including as well as excluding the 28 assumed rectosigmoid cases.

Whether a patient was invited was related to tumor status and therapy. Participating cases did not differ significantly in demographic factors from all cases who could have been included in the study. As a result of differences in invitation rates, participants had more Dukes' A or B tumors than the total group of eligibles, which might benefit the quality of the exposure data. More severe cases might have changed their diet as a result of the disease, which might make it more difficult to

recall previous dietary patterns. Furthermore, nausea from chemotherapy, which occurs more frequently among patients with Dukes' C and D tumors, might influence both appetite and the ability to talk about food for two hours or more.

Recruitment of controls

Rationale
In this study controls were sampled via the general practitioner of the interviewed case. This procedure was used to increase response rate and efficiency and to ensure a justifiable link to the source population. As observed in a previous case-control study on breast cancer, sampling of controls from municipal population registries has the disadvantage of relatively low response rates (55% among women). Additionally, the latter sampling method has a practical disadvantage since the source population of the cases is difficult to define before cases are recruited; this is especially true since not all hospitals in a given area are willing or able to cooperate in a study. Furthermore, the increased awareness of privacy regulations introduces practical difficulties in sampling from municipal registries.

   To investigate the advantages and disadvantages of this GP-mediated procedure as compared to the municipal registry method, a age- and gender-stratified sample was recruited from municipal registries of four representative towns.

Procedures for control recruitment
Three persons of the same gender and approximately the same age (± 5 years) as the case were randomly selected by the general practitioner's of the case. For each case, the GP received a standardized form with three-letter strings, randomly obtained from the local telephone directory to initiate the search for controls in his or her own database; for each string, the first subject who matched the eligible criteria was send a standard letter of invitation, including a reply form to the investigator. After two weeks, each non-responder received a reminding letter. The first or second responder was included in the study.

   Since GPs often have separate databases for privately and non-privately insured patients, they were asked to draw two non-privately insured and one privately insured patients from their database(s), which resembles the ratio in the general population.

Cooperation of general practitioners
Of the 264 approached general practitioners (GPs), 67% were willing to cooperate. If the GP refused cooperation a gender-matched control who resembled the patient as much as possible concerning age, gender and urbanization grade and region was

selected from controls sampled by other GPs. If not available (5%), a control sampled from the municipal registry of the town involved was chosen.

GPs were asked to complete a short questionnaire inquiring about the way the three control subjects were sampled; 100% replied and reported that they had followed the sampling instructions.

Cooperation of municipal registries

The municipal registries of four suburban and two urban towns were invited to draw a age-stratified sample of 50 to 100 inhabitants. The required number of controls to be sampled from each five-year age category was calculated on the basis of colon cancer incidence figures.

Each registry was willing to cooperate, but for budgetary reasons it was decided to draw samples from three suburban and one urban town only. For privacy reasons, the urban registry conducted the mailing themselves.

Participation of GP-mediated controls

Of the 520 GP-mediated controls invited, 79% returned the reply form (60% before a reminding letter was sent), while 75% filled out the short questionnaire and 57% was willing to participate in the interview. Age was related to response: persons under 40 where more inclined to participate in the interview (Table 4.4). Men and women did not differ in readiness to fill out the questionnaire, although women tended to be more inclined to participate in the interview. Response rates were higher in urban regions. From the 294 GP-mediated controls who were willing to be interviewed, a subsample was selected which resembled the case-population as closely as possible according to age, gender, region and urbanisation grade.

In total, 266 controls were interviewed. Of those interviewed, five did not meet the inclusion criteria (3 not western European, 1 previous cancer and 1 Crohn's disease) and one interview was not completed.

Of the 259 controls participating in the interview 95% were recruited through the GP-mediated procedure, while 5% were sampled from municipal registries.

The age and gender distribution of the 259 eligible participating controls resembled closely the distribution of those invited (Table 4.5). The participants were likely to be more urban citizens and were more often privately insured. In multivariate logistic regression analysis, with participation being the dependent variable, both urbanization grade and health insurance contributed significantly to the model.

The proportion of privately insured controls closely resembles the proportion in the general population, which is about one third.

Table 4.4   Response rates, stratified according to demographic variables, of invited population controls (n = 520).[$]

	Replied[1] (%) (n = 409)	Filled out short questionnaire[2] (%) (n = 389)	Willing to participate in interview[3] (%) (n = 294)	Participated in interview[4] (%) (n = 259)[$$]
Overall response (%)	79	95	76	88
Demographic variables				
age < 40 years	89	96	91	60
40-49	73	92	72	90
50-59	77	98	79	86
60-69	79	95	75	95
> 69	82	94	70 *	88 *
gender male	78	95	74	88
female	80	95	77	88
region urban	82	94	77	90
rural	73 *	97	74	85
health insurance				
private	82	95	79	90
non-private	76	95	74	88
unknown[$$$]	100	96	86	100

*   heterogeneity in response (p < 0.05).
[1]   replied as a percentage of invited.
[2]   short questionnaire as a percentage of replied.
[3]   willing to participate as a percentage of short questionnaire.
[4]   participated as a percentage of willing to participate.
[$]   recruited via general practitioners.
[$$]   95% recruited via general practioners, 5% sampled from municipal registries.
[$$$]   category 'unknown' not included in analyses of heterogeneity.

Municipal and GP-mediated controls

Of the 424 invited controls sampled from municipal registries, 71% filled out the short questionnaire as compared to 76% of the suburban and urban GP-mediated controls. Table 4.6 presents the characteristics of municipal controls as compared to suburban and urban GP-mediated controls. Selection might have occurred if GPs did not adhere strictly to the protocol and were more prone to invite persons who regularly visit their doctor. Consistent with the reported adherence to the sampling protocol (see 'Cooperation of general practitioners'), the frequency of visits was similar for GP-mediated and municipal controls (22% versus 25% more than twice a year). Age, body mass index, gastro-intestinal complaints, changes of diet because of gastro-intestinal complaints, the consumption of whole-wheat bread, meat and unfermented dairy products did not differ appreciably between the groups of

controls recruited via these different procedures. Men tended to be more willing to cooperate when invited by their GP. Municipal controls who filled out the questionnaire were more likely to be privately insured; the proportion of private insured (57%) greatly exceeded the proportion in the general population (33%).

Table 4.5   Distribution of demographic characteristics of invited population controls[$] and the actual eligible population controls participating in the home interview.

		Invited (n = 520)	Actual participants in interview (n = 259)
Demographic variables			
age (%)	< 40 years	5	5
	40-49	12	11
	50-59	25	26
	60-69	38	40
	69-75	20	19
gender (% male)		54	53
region (% urban)		62	66*
health insurance (% private)		34	38**

*	differs from non-participants (58%; p = 0.05).
**	differs from non-participants (30%; p = 0.05).
[$]	95% recruited via the GP-mediated procedure, 5% sampled from municipal registries.

Fermented dairy products were consumed more frequently by the municipal controls. After stratification for health insurance this difference was no longer important. However, in multivariate analyses with type of control (GP-mediated or municipal) being the dependent variable, gender, insurance and the consumption of fermented dairy products contributed independently.

In summary, comparing the two procedures of control recruitment, the GP-mediated procedure has two advantages: response of invited controls is slightly higher and, more importantly, the distribution of privately insured people is comparable to that in the general population. Since GPs adhered to the protocol and the numbers of GP visits are similar for the controls recruited by both procedures, no important selection of controls does seem to occur using the GP databases. It can be concluded that the GP-mediated procedure is a reliable method for control recruitment and that it might be preferable to the municipality method used in previous studies.

Implications of control recruitment

The participating controls were similar to those invited in age and gender, which implies a close resemblance to the case group. Participating controls lived more frequently in urban areas and were more often privately insured than the total group

participating controls, odds ratios were adjusted for socio-economic level, in data
analysis.

As anticipated on, the response of urban GP-mediated controls was higher than
that of the municipal controls (Table 4.6). The controls recruited by these two
procedures differed in the proportion of men, proportion of privately insured people
and the consumption of fermented dairy products. According to the proportion of
men and those privately insured, the GP-mediated controls appear to resemble the
general population more closely.

Table 4.6   Characteristics of (sub)urban population controls recruited via different procedures
             who completed the short questionnaire.

Variables	Recruitment procedure	
	via general practitioner (n = 249)	via population registry (n = 301)
Response		
complete refusers (%)	18	26
short questionnaire (%)	78	71
Demographic variables		
age (years, mean ± SD)	62 ± 10	63 ± 10
gender (% male)	54	44
health insurance (% private)$	36	57
Health indicators		
body mass index (mean ± SD)	25 ± 3	25 ± 4
visits to general practitioner (% more than twice a year)$$	22	25
gastro-intestinal complaints (% yes)	17	16
changes in diet because of gastro-intestinal complaints (% yes)	5	9
Dietary habits		
meat (% every day)	47	50
whole-wheat bread (% more than 5 slices per day)	16	17
dairy products (% in highest category)	32	40
fermented dairy products (% in highest category)	29	37*

*   p = 0.05.
$ percent unknown: 4 in both categories.
$$ percent unknown: 13 and 7 respectively.

Indicators of non-response

The short 'non-response' questionnaire was completed by each case and control
participating in the home interview and by only 30 of 122 non-participating cases
and 95 of 226 controls not willing to participate. Thus, only a small fraction of the

non-participants completed the questionnaire. Of the 30 eligible non-participating cases, 13 refused participation for medical or psychological reasons, 10 did not want to participate for unknown reasons, while 7 did not fill out the reply form at all. Reasons for non-participation for the other 302 non-participants remain unknown.

Table 4.7   Health indicators and dietary habits of those eligible colon cancer cases and controls who only filled out the short questionnaire and those additionally participating in the home interview.

Variables	Colon cancer cases		Population controls	
	short questionnaire only (n = 30)	home interview (n = 200)	short questionnaire only (n = 95)	home interview (n = 259)
Health indicators				
body mass index (mean ± SD)	23 ± 4	25 ± 4	25 ± 3	26 ± 4
gastro-intestinal complaints (% yes)	37	32	18	16
dietary changes because of gastro-intestinal complaints (% yes)	17	9	6	9
Dietary habits				
meat (% every day)	57	62	52	45
whole-wheat bread (% more than 5 slices a day)	7	24	17	19
dairy products* (% highest category)	40	31	40	35
fermented milk (% highest category)$^{\$}$	53	29*	24	30

$^{\$}$ excluding cheese.
* $p < 0.05$.

Of the 95 non-participating controls 1 deceased, 4 refused participation for medical reasons, 6 replied not to have time to participate, while 79 did not give a reason and 13 did not fill out the reply form. For the other 131 non-participants the reasons for non-participation remain unknown as well.

Thus, it remains unknown to what extent the non-participants who filled out the short questionnaire are representative of the total group of non-participants as far as diet is concerned. Nevertheless, in the absence of other information, the above comparison may suggest the direction of a potential selection in participation.

Table 4.7 presents the distribution of health indicators and relevant dietary habits for those eligibles interviewed (200 cases and 259 controls) compared to those who only filled out the short questionnaire.

Cases with gastro-intestinal complaints and those who said they had changed their diet because of these complaints participated less frequently in the interview, however this difference was not statistically significant (Table 4.7). Body mass index, meat consumption and the use of dairy products were not strongly related to participation in cases and controls, while case participants ate more whole-wheat bread and less fermented dairy products (Table 4.7). Besides chance, these differences might be explained by a higher socio-economic status of the participating cases or by the fact that more male cases participated in the interview. In data analysis, odds ratios were adjusted for gender and indicators of socio-economic status.

The way in which the above findings bias study results depends not only on cases, but also on the potential exposure-related selection among controls. Cases participating in the interview consumed less fermented dairy products than the eligible cases. Since, participating controls consumed fermented dairy products more frequently, the odds ratio calculated from the data may be stronger than the underlying true odds ratio.

4.2   Collection and validity of the data

Data collection involved a short questionnaire, a home interview and collection of toenail clippings.

Short questionnaire
The short questionnaire was enclosed in the letter of invitation. This questionnaire inquired about major dietary patterns and life-style variables of interest. The objective of this short inquiry was to be able to compare those willing to participate in the home interview with those not willing and thus to investigate if a certain selection was introduced by non-response to the request for a home interview. The same questionnaire was used to compare controls recruited by the GP-mediated method and the municipal registry procedure.

Home interview
Participating cases and controls were interviewed at their homes by one of seven trained dieticians. The questionnaire consisted of a dietary history part and questions considering life-style, medical history and family history of colorectal cancer. The home interview took about three hours per participant, two hours of which were taken by the dietary history part.

The interview started with questions regarding special dietary regimens (e.g. vegetarianism) diets gastro intestinal complaints and related diet

It continued with a structured dietary history part inquiring about the consumption of 289 food items from 19 food groups consumed in the preceding year. For cases the year preceding complaints or diagnosis was asked for. The hierarchically structured questionnaire was divided into five sections corresponding with the national pattern and starting with the most intensive part: the main meal (appetizers, main dishes), followed by breakfast and lunch, desserts, (non)alcoholic drinks and snacks. To be able to estimate serving sizes, frequently used household utensils and cups were measured.

Additionally, for products of special interest to this study, changes in the habitual use as compared to five years earlier were asked for.

At the end of the interview, questions related to life-style (e.g. supplement use, smoking habits, sunlight exposure) were asked together with questions on previous or current illnesses, previous or current use of medicin and family history of colorectal cancer. Furthermore, the participant was weighed.

To account for seasonal variation in food habits, cases and controls were interviewed in the same season. To account for differences between interviewers, each interviewer visited a similar number of cases and controls.

Food items were coded according to the Dutch National Food Table.[5] To minimize errors, the dietary history questionnaire was entered into the computer using a specially designed computer program which included range-checks and cross-checks. For each food item default values for frequency of use, number of servings, serving sizes and food code were pre-coded and could be skipped, copied or changed when a different value was required. Checks on the internal consistency of the frequency of consumption of various meal components with the total meal pattern were conducted. When frequencies did not match, a correction factor was automatically calculated and added to each item in the data file.

Alterations of the questionnaire during the interview period

Besides the main hypothesis, other issues were planned to be studied, which sometimes needed an extention or alteration of the original questionnaire. Not all of the new additions turned out to be successful. Especially with the assessment of height, physical activity and the combined consumption of fermented dairy products and nitrate-rich foods practical difficulties were encountered.

In the beginning of the study, both height and weight of the participants were determined. However, measuring height turned out not to be practically feasible in the home interview and was therefore considered unreliable.

The section involving the assessment of physical activity was altered in the general questionnaire on life-style. This part consisted of questions taken from a validated questionnaire[6] but did not appear to be feasible in this case-control

interview setting. Originally the physical activity questionnaire was designed to inquire about current behaviour. In the case-control study it should refer to the previous year and for cases to the period before diagnosis or complaints, which could not be reliably answered by the participants. To our knowledge, no validated questionnaire on physical activity based on a retrospective design was available during the conduct of the study.

The combined use of fermented dairy products (cheese, buttermilk, yogurt) and nitrate-rich vegetables was assessed in the original questionnaire. The questions appeared too difficult to ask and to answer and were skipped from the questionnaire.

Participants in the home interview were asked to collect toenails. It was originally planned to analyse these toenails for selenium status. However, considering the costs and the findings of recently conducted epidemiological studies, which did not support the hypothesized inverse association between selenium and colon cancer,[7] it was decided not to conduct such analyses.

Validity of dietary data and information bias
The structure of the dietary history questionnaire was similar to that used by van 't Veer and co-workers in a previous case-control study on breast cancer.[8] The internal consistency checks and correction procedures are similar to those used in the breast cancer case-control study and to those used in the semi-quantitative food frequency questionnaire of the Netherlands Cohort Study on diet and cancer.[9]

Besides being interviewed with the dietary history questionnaire, 114 controls who participated in the previous case-control study on breast cancer also filled out the food frequency questionnaire used in the Netherlands Cohort Study.[10] In the cohort study the food frequency questionnaire was validated against a 3-day record repeated three times.[9] With respect to macro- and micronutrients, the dietary history was related to both the food frequency questionnaire and the dietary record. Coefficients of correlation were similar for both comparisons.[10]

To study whether the food-frequency questionnaire used in the cohort study could be used in a case-control design, this questionnaire was additionally completed by 77 cases and 80 controls participating in the dietary history interview of our case-control study. These data will be analysed at a later stage.

To assess significance of differences between interviewers two-way analysis of variance was conducted. The difference in intake of energy and calcium (and therefore dairy product consumption) between cases and controls did not differ markedly between interviewers.

Total energy intake was significantly higher among cases, which is supported by other case-control studies on colorectal cancer.[11] This might suggest that a high

energy intake increases the risk of colon cancer but, more likely, it might point to the fact that cases overestimate their habitual intake. Both explanations, however, require nutrient intakes to be adjusted for total energy intake in data analysis.

One case was excluded from the analysis since the interviewer evaluated the dietary history data as unreliable and total energy intake exceeded 25,000 kJ.

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Abstract

To examine whether the consumption of fermented dairy products or the dietary intake of calcium decreases colon cancer risk, a case-control study was conducted in the Netherlands. Dietary patterns were assessed in detail among 232 cases and 259 controls (for cases before diagnosis or symptoms occurred) using a structured dietary history questionnaire.

After adjustment for potential confounding variables, consumption of fermented dairy products, hard cheese and unfermented dairy products was not significantly associated with risk of colon cancer: an odds ratio (OR) of 1.1 (95% confidence interval (CI) = 0.6-1.8) was found for individuals consuming more than one serving of fermented dairy products per day as compared to those consuming less than 10% of one serving a day. Adjustment for dietary calcium attenuated the associations.

Total dietary calcium was positively but non-significantly associated with colon cancer risk after adjustment for age, gender, urbanization level and total energy intake (OR highest vs. lowest quartile of intake = 1.4; 95% CI = 0.9-2.4). Additional adjustment for a positive family history of colorectal cancer, cholecystectomy and energy-adjusted intake of total fat, dietary fiber, vitamin C and alcohol increased the association (OR highest vs. lowest quartile of intake = 1.8; 95% CI = 1.1-3.1). No differences were observed between calcium from fermented and calcium from unfermented dairy sources.

The observed associations for fermented dairy products and dietary calcium differed between men and women: positive significant associations were observed in men, while in women non-significant inverse associations were found.

These results do not support the hypothesis that an increased intake of commercially available, commonly used fermented dairy products or dietary calcium decreases the risk of colon cancer.

Introduction

Since colon cancer incidence and mortality are still relatively high in westernized countries and new treatment strategies are not expected to be available in the near future, preventive measures seem to be essential in controlling this disease. Minor changes in dietary patterns might offer substantial possibilities for prevention.[1] Already in 1908, Eli Metchnikoff propagated an increased consumption of fermented foods, especially milk products such as yogurt, cultured milk and cheese, to suppress colon carcinogenesis.[2] Recent animal and human experimental studies support the idea that certain strains of lactic acid bacteria might decrease colon cancer risk.[3] Several potential mechanisms of action have been suggested: decrease in activity of faecal enzymes thought to be involved in colon carcinogenesis by certain strains of *Lactobacillus*,[3-5] suppression of tumors through enhancement of the immune response,[6,7] cellular uptake of mutagenic compounds,[8] and antioxidative properties of specific strains.[9]

Moreover, fermented dairy products are an important source of calcium in a westernized diet. Calcium is suggested to decrease colon cancer risk through a decreased cell proliferation induced either by cell differentiation[10] or by binding of toxic bile acids and free fatty acids in the colonic lumen.[11] Milk products are rich in phosphate, which has been hypothesized to inhibit the antiproliferative effect of calcium,[11] while in vitro studies have shown that bile acids are precipitated by insoluble calcium phosphate.[12]

It is still unknown whether generally consumed and commercially available fermented dairy products exert an anticarcinogenic or antimutagenic effect. Few epidemiological studies have addressed the association between consumption of fermented dairy products (excluding cheese) and colon cancer risk.[13-15] Two population-based American case-control studies did observe an inverse association with the consumption of cultured milk (yogurt and buttermilk)[14] and yogurt[15] after adjustment for potential confounding factors.

The association with the intake of dairy products in general and/or calcium is studied more often in case-control and cohort studies, but results remain inconsistent.[15-24] Some studies suggest stronger associations for women than for men.[17,24,25]

In contrast with the countries in which these studies have been conducted, the Netherlands is known for its high per capita consumption of fermented dairy products (e.g. 18.9 kg per year vs. 2.1 kg per year in the US)[26] and the substantial heterogeneity of its consumption patterns. In previous Dutch case-control studies on

breast cancer[27] and cancer of the exocrine pancreas[28] inverse associations with fermented dairy product consumption were observed.

We studied the association between fermented dairy products, the intake of calcium from different dairy and non-dairy dietary sources, and colon cancer risk among men and women in a case-control study using a structured dietary history questionnaire to assess usual fermented dairy product consumption and other dietary variables in greater detail.

Subjects and methods

A case-control study of colon cancer was conducted in the Netherlands between 1989 and 1993. Dutch-speaking men and women of west European origin, up to the age of 75 at diagnosis, who resided in the central or eastern parts of the Netherlands and did not have a history of cancer, ulcerative colitis or Crohn's disease were eligible for this study.

Cases
Cases were defined as newly diagnosed and histologically confirmed patients with adenocarcinomas of the colon (ICD-O 153). They were recruited as soon as possible after diagnosis by their medical specialists in 14 regional hospitals. The cancer registries of the central and eastern parts of the Netherlands were used as a check of completeness and provided additional clinical and pathological information. For 28 cases, the cancer registry defined the location of the tumor as rectosigmoid while according to the surgeon the tumor was located in the colon. Analyses were conducted both including and excluding these cases.

Of all eligible cases diagnosed in the cooperating hospitals, 47% were invited to participate. Cases invited did not differ importantly from those not invited in age, gender, urbanization level and site distribution. Cases who were not invited had a worse prognosis according to Dukes' status and more frequently underwent radiotherapy and chemical therapy. Of those invited, 60% agreed to be interviewed. Except for Dukes' status, the distributions of age, gender, tumor site and therapies of the interviewed cases were comparable to those of all eligible cases. All participants and 23% of the non-participants completed a short questionnaire which included questions on the most relevant dietary and life-style factors. Cases participating in the interview consumed less fermented dairy products than those non-participants who filled out the questionnaire. The two groups did not differ markedly in their consumption of unfermented dairy products. The interval between diagnosis and interview was about three to six months.

Controls
Population controls, of similar age (5-year intervals), gender, region and degree of urbanization as the cases, were recruited randomly by the general practitioners of the participating cases. Of the 520 controls invited, 57% agreed to participate in the interview. Participation was higher among younger people and those living in urban areas. Dietary and life-style patterns did not differ importantly between those who participated in the interview and those who refused to participate but completed the non-response questionnaire (42% of non-participants).

Questionnaire
Trained dieticians conducted personal interviews in the participant's home. Each interviewer visited both a case and a control of the same age, gender, region and urbanization level. Interviewing cases and controls was balanced over seasons to account for seasonal fluctuations in food patterns. The interview consisted of a dietary history part and questions considering life-style, medical history and family history of colorectal cancer.

The hierarchically structured part[29] was divided into five sections according to national habits and starting with the most intensive part, the main meal (appetizers, main dishes), followed by breakfast and lunch, desserts, (non-)alcoholic drinks and snacks. The questionnaire covered the complete dietary pattern. The consumption frequency per month in the preceding year (in cases before diagnosis or symptoms), number of months duiring which the item was used, number of portions per consumption and portion size of 289 food items from 19 food groups were inquired. The questionnaire included all major dairy products consumed in the Netherlands. To be able to estimate portion sizes, frequently used household utensils and cups were weighed.

To minimize errors, the dietary history questionnaire was entered into the computer using a specially designed computer program which included range-checks and cross-checks. Checks on the internal consistency of the frequency of consumption of various meal components with the total meal pattern were conducted. Frequencies of individual items were corrected automatically in order to agree with the frequency of the total food group.

Average daily intake of dietary calcium and other nutrients was calculated using the Dutch National Food Table.[30] Intake of nutrients was adjusted for energy intake by regression analysis[31] for men and women separately.

To exame the stability of food habits, changes in habits as a result of gastro-intestinal complaints were inquired.

The non-dietary part of the interview included questions on socio-demographic indicators (e.g. age, education, occupation, urbanization level), medical history (e.g.

cholecystectomy), supplement use, family history of colon cancer, reproductive factors (number of children and age at first birth) and, among female participants, on menstrual history and use of oral contraceptives. Occupation was used as a measure of socio-economic status (SES); for married women (including divorcees and widows), SES was based on their (former) husband's occupation.

Data analysis

In this analysis emphasis was on three groups of dairy products: fermented dairy products (buttermilk, yogurt, curds and kefir); hard cheese (i.e. Gouda cheese) and unfermented dairy products (whole-fat milk, skim/low-fat milk, custards, evaporated milk and porridge). The intake of dairy products is expressed in grams per day. Categories or quartiles of dairy product use and dietary calcium intake were based on the distribution among controls. The lowest category or quartile of intake was used as the reference. Different sources of dietary calcium were explored. Since these analyses focussed primarily on calcium from dietary sources, supplemental calcium (alone, with vitamin D, or as multivitamin supplement) was examined as a confounder and coded as a dichotomous variable (use yes or no).

A high phosphorus intake relative to the intake of calcium was evaluated using quartiles of the ratio of calcium to phosphorus with the lowest quartile as the reference.

Odds ratios (OR) as estimators of relative risks and approximate 95% confidence intervals (95% CI) were calculated by unconditional maximum likelihood estimation using BMDP software.

Variables for which frequency matching was conducted (age, gender and urbanization level) were included in all statistical models. Total energy intake was additionally included to adjust for differential overreporting.

To evaluate the potential confounding effects of energy-adjusted intake of fat, carbohydrates, dietary fiber and vitamin C, smoking habits, calcium supplement use, socio-economic status, cholecystectomy and family history of colorectal cancer, these covariates were added to the logistic regression model one at a time. Decisions on which covariates to include in the final models were based on: 1) literature and previous studies,[32] 2) biological plausibility, 3) whether the regression coefficient of the primary independent variable changed by 10% or more after addition of the potentially confounding variable, and 4) whether the covariate entered the model at p < 0.10. A priori it was decided to adjust for nutrients instead of food groups.

We tested for overall trends by using the median of each quartile as a continuous variable. Since no apparent differences were found in the analyses after exclusion of

potential rectosigmoid cases (data not shown), results are presented including these cases.

Results

Table 5.1 shows various characteristics of controls according to high and low intake of fermented dairy products and dietary calcium. Those in the highest quartile of fermented dairy product consumption (mean intake 391 g per day excluding cheese) did not differ importantly in energy intake and energy-adjusted intake of dietary calcium, dietary fiber and alcohol from those in the lowest quartile (mean intake 4 g per day).

Energy-adjusted intake of fat was higher among those with the lowest intake, while vitamin C intake was higher among the most frequent consumers. Women did consume fermented dairy products more frequently than men and those with the highest intake were younger. Body mass index and a family history of colorectal cancer did not differ appreciably between the two categories of intake. Although not significantly different, cholecystectomy prevalence was higher, socio-economic level was higher, calcium phosphate ratio was higher and calcium supplements were used more frequently among those with the highest intake. The highest category of intake included less current smokers.

Dietary calcium intake was not strongly associated with age, gender, energy-adjusted intake of fat and alcohol, body mass index, cholecystectomy, calcium supplement use or history of colorectal cancer. Total energy intake and energy-adjusted intake of dietary fiber and vitamin C were higher and calcium/phosphorus ratio was higher among those with the highest dietary calcium intake. Those in the highest quartile of intake had a higher socio-economic level and less persons currently smoked.

Table 5.2 illustrates the distribution of colon cancer cases and controls according to dairy product consumption and dietary calcium intake. The median intake of fermented dairy products, hard cheese, unfermented dairy products and total dietary calcium was higher among cases.

In Table 5.3 odds ratios of colon cancer are presented according to dairy product consumption. The consumption of fermented dairy products, hard cheese and unfermented dairy products was not significantly associated with colon cancer risk adjusted for age, gender, urbanization level and total energy intake. For the most frequently used fermented dairy products, yogurt and buttermilk, a non-significant positive and inverse association was observed respectively. For neither whole milk nor skim milk or low-fat milk important associations were observed.

Table 5.1 Characteristics of controls (n = 259) according to highest and lowest quartiles of fermented dairy product consumption and calcium intake.

Variables	Fermented dairy products (excl. cheese)[$]		Calcium	
	Q1	Q4	Q1	Q4
Age ± SD	63.9 ± 9.1	60.6 ± 8.7 **	62.4 ± 9.9	62.0 ± 9.2
Gender (% men)	66.7 ± 5.8	32.8 ± 5.8 **	50.8 ± 6.2	60.9 ± 6.1
Dietary factors (mean ± SD)				
Fermented dairy (excl. cheese) (g/day)	4 ± 7	391 ± 171 **	82 ± 95	222 ± 241 **
Hard cheese (g/day)	35 ± 29	37 ± 26	18 ± 12	63 ± 38 **
Unfermented dairy (g/day)	284 ± 223	211 ± 272 *	122 ± 108	488 ± 320 **
Dietary calcium (mg/day)[†]	1,150 ± 355	1,436 ± 449	905 ± 180	1,722 ± 412 **
Total energy (kJ/day)	9,062 ± 2,527	9,023 ± 2,893	8,047 ± 2,200	11,125 ± 2,973 **
Total fat (g/day)[†]	108 ± 24	92 ± 22 **	103 ± 23	101 ± 28
Dietary fiber (g/day)[†]	28 ± 8	30 ± 7.7	26 ± 7	31 ± 9 **
Vitamin C (mg/day)[†]	104 ± 54	135 ± 55 **	95 ± 40	132 ± 60 **
Alcohol (g/day)[†]	16 ± 20	11 ± 16	17 ± 18	12 ± 18
Calcium/phosphorus ratio[†]	0.7 ± 0.1	0.8 ± 0.1 **	0.6 ± 0.1	0.8 ± 0.1 **
Medical and life-style factors				
Body mass index (mean ± SD)	26.6 ± 4.0	25.8 ± 4.0	26.5 ± 4.0	26.0 ± 3.7
Cholecystectomy (% ± SE[‡])	3.0 ± 2.1	6.3 ± 3.0	4.7 ± 2.6[1]	6.3 ± 3.0
Family history of colorectal cancer (%±SE[‡])	11.1 ± 4.0[3]	9.8 ± 3.8[3]	11.5 ± 4.1[4]	9.8 ± 3.8[3]
SES (% blue collar ± SE[‡])	45.5 ± 6.1	35.5 ± 6.1	51.6 ± 6.2	22.6 ± 5.3[1] **
Current smokers (% ± SE[‡])	42.2 ± 6.2[2]	28.1 ± 5.6 *	51.6 ± 6.2[1]	26.6 ± 5.5 **
Calcium supplement users (% ± SE[‡])	15.4 ± 4.5	25.0 ± 5.4	23.1 ± 5.2	17.2 ± 4.7

[$] buttermilk, yogurt, curds, kefir, other fermented milk drinks, sour cream.
[*] different from Q1; p ≤ 0.1.
[**] different from Q1; p ≤ 0.05.
[1,2,3,4] 1,2,3,4 missing values respectively.
[†] adjusted for total energy intake through regression analysis.
[‡] instead of SD, SE = $\sqrt{(p(1-p)/n)}$ is given for proportions.

Table 5.2 Distribution of colon cancer cases (n = 232) and population controls (n = 259) according to average daily consumption (g) of dairy products and daily intake of calcium (mg).

	cases median	(P25-P75)	controls median	(P25-P75)
Total fermented dairy products (excl cheese)*	107	(17-229)	101	(22-242)
Yogurt	34	(33-100)	26	(0-91)
Buttermilk	0	(0-101)	0	(0-113)
Hard cheese	37	(20-57)	31	(19-49)
Other cheeses	0	(0-4)	0	(0-4)
Total unfermented dairy products*	223	(100-473)	205	(81-368)
Whole milk	0	(0-0)	0	(0-0)
Skim/low-fat milk	66	(0-258)	43	(0-252)
Total dietary calcium	1298	(1021-1593)	1209	(1010-1480)
From fermented dairy products	527	(336-729)	485	(333-683)
From unfermented dairy products (incl. cheese)	319	(158-576)	328	(170-530)
From non-dairy products	335	(280-386)	345	(296-402)

* buttermilk, yogurt, curds, sour cream, kefir, other fermented milk drinks.

** whole milk, skim/low fat milk, chocolate milk, other milk drinks, evaporated milk, custards, porridge.

Additional adjustment for a family history of colorectal cancer, cholecystectomy, energy-adjusted intake of fat, dietary fiber, vitamin C and alcohol increased the odds ratios for total fermented dairy products, yogurt and unfermented dairy products which was especially due to the inclusion of vitamin C, dietary fiber and cholecystectomy in the model.

However, none of the category specific rates trends were statistically significant. Socio-economic status, calcium supplement use and smoking status were not included in the models since they did not further affect the estimates. Adjustment for dietary calcium diminished the odds ratios (ORs highest vs. lowest quartile for fermented and unfermented dairy products were 0.90 and 1.26 respectively).

Table 5.4 presents the odds ratios for energy-adjusted dietary calcium intake and calcium from different dietary sources. In multivariate models total dietary calcium was positively associated with colon cancer risk. The energy-adjusted intakes of vitamin C and alcohol acontributed mainly to the increased odds ratio. Neither

adjustment for vegetables instead of vitamin C and dietary fiber nor adjustment for meat changed the estimates appreciably.

Table 5.3   Odds ratios of colon cancer for categories of dairy product consumption.

Products (g/day)		Number of		Odds ratios (95% CI)		$\chi^{2\$}$
		Cases	Con-trols	Adjusted for age, urbanization level, gender, total energy	Multivariate*	(p)
Fermented dairy	≤ 22	66	66	1.00	1.00	
products	22-101	48	65	0.74 (0.44-1.24)	0.84 (0.49-1.43)	0.27
	101-242	62	64	1.00 (0.61-1.66)	1.16 (0.69-1.96)	(0.60)
	> 242	56	64	0.86 (0.51-1.44)	1.06 (0.61-1.82)	
Buttermilk	non-users	124	132	1.00	1.00	0.06
	≤ 113	54	63	0.94 (0.60-1.47)	0.99 (0.62-1.58)	(0.80)
	> 113	54	64	0.89 (0.56-1.39)	0.94 (0.59-1.50)	
Yogurt	non-users	76	88	1.00	1.00	3.11
	≤ 26	29	42	0.84 (0.47-1.50)	0.86 (0.47-1.55)	(0.08)
	26- 91	65	65	1.20 (0.74-1.93)	1.49 (0.90-2.45)	
	> 91	62	64	1.16 (0.71-1.88)	1.49 (0.89-2.49)	
Hard cheese	≤ 19	55	65	1.00	1.00	0.57
	19-31	44	65	0.90 (0.52-1.52)	0.92 (0.53-1.62)	(0.45)
	31-49	58	67	1.05 (0.63-1.76)	1.08 (0.63-1.83)	
	> 49	75	62	1.21 (0.72-2.03)	1.18 (0.69-2.01)	
Unfermented	≤ 81	42	65	1.00	1.00	1.12
dairy products	81-205	67	65	1.44 (0.85-2.46)	1.59 (0.92-2.76)	(0.29)
	205-368	46	65	0.96 (0.55-1.69)	1.01 (0.57-1.81)	
	> 368	77	64	1.36 (0.79-2.36)	1.59 (0.89-2.85)	
Milk whole	non-users	180	196	1.00	1.00	0.02
	≤ 50	16	32	0.46 (0.24-0.89)	0.41 (0.21-0.82)	(0.88)
	> 50	36	31	1.00 (0.58-1.73)	0.95 (0.54-1.68)	
Skim milk/	non-users	70	85	1.00	1.00	0.03
low-fat milk	≤ 185	90	88	1.38 (0.88-2.15)	1.44 (0.91-2.29)	(0.85)
	> 185	72	86	1.01 (0.64-1.60)	1.15 (0.71-1.84)	

$\$$ χ^2 for trend multivariate model, p-value in parentheses.
* Adjusted for age, gender, urbanization level, family history, cholecystectomy, total energy intake, energy-adjusted intake of fat, dietary fiber, vitamin C and alcohol.

Table 5.4   Odds ratios of colon cancer for quartiles of calcium intake.

Nutrient	Level of consumption				χ^{2*} (p)	Continuous per
	Q1	Q2	Q3	Q4		200 mg
Total dietary calcium						
mg/day	≤ 1,010	1,010-1,209	1,209-1,480	> 1,480		
number of cases/controls	57/65	45/65	50/65	80/64		
OR 1**	1.00	0.92	0.97	1.42	2.09 (0.15)	1.05 (0.96-1.14)
OR 2***	1.00	0.94	1.15	1.81	5.36 (0.02)	1.09 (0.99-1.20)
95% CI		(0.54-1.63)	(0.66-2.00)	(1.05-3.12)		
Calcium from fermented dairy products$						
mg/day	≤ 333	333-485	485-683	> 683		
number of cases/controls	57/64	50/65	60/65	65/65		
OR 1**	1.00	0.99	1.17	1.18	0.58 (0.45)	1.05 (0.93-1.18)
OR 2***	1.00	1.03	1.28	1.26	1.00 (0.32)	1.08 (0.95-1.22)
95% CI		(0.60-1.76)	(0.74-2.20)	(0.74-2.16)		
Calcium from unfermented dairy products						
mg/day	≤ 170	170-328	328-530	> 530		
number of cases/controls	63/64	57/66	50/65	62/64		
OR 1**	1.00	1.15	0.97	1.00	0.05 (0.82)	1.06 (0.95-1.18)
OR 2***	1.00	1.25	1.09	1.10	0.01 (0.94)	1.08 (0.96-1.22)
95% CI		(0.73-2.15)	(0.63-1.91)	(0.63-1.91)		
Calcium from non-dairy products						
mg/day	≤ 296	296-345	345-402	> 402		
number of cases/controls	74/65	52/64	62/66	44/64		
OR 1**	1.00	0.74	0.82	0.56	3.97 (0.05)	0.58 (0.36-0.91)
OR 2***	1.00	0.77	0.89	0.69	0.91 (0.34)	0.68 (0.37-1.27)
95% CI		(0.45-1.31)	(0.51-1.54)	(0.36-1.32)		

* χ^2 for trend.
$ fermented milk and cheese.
**   OR 1: Adjusted for age, gender, urbanization level, total energy intake.
***   OR 2: Adjusted for age, gender, urbanization level, family history, cholecystectomy, total energy intake, energy-adjusted intake of fat, dietary fiber, vitamin C and alcohol.

participating and non-participating controls who completed the questionnaire. However, these findings need to be interpreted with caution since only about 25% of the non-participating cases and 45% of the non-participating controls filled out the non-response questionnaire.

In the Netherlands as well as the US,[32] the consumption of fermented dairy products and calcium is related to a healthier life-style and a higher socio-economic status. If selection of a relatively healthy, health-conscious control group would have occurred, we would have observed no association rather than a positive association for calcium. We recruited the control group via the databases of the general practitioners of the cases interviewed. When we compared this control group with the general population we found a comparable distribution of socio economic status. Another control group recruited via the municipal registries included more persons with a higher socio-economic status. Thus, an inverse association might have been erroneously found when municipality controls had been used as in previous studies.[27,28] In conclusion, information and selection bias are not likely to explain our findings.

The absence of an inverse association cannot easily be explained by insufficient contrast in this population with a relatively high and heterogeneous consumption of fermented and unfermented dairy products.

The significant positive association with dietary calcium might be the results of our adjustment strategy. Excluding vitamin C from the multivariate model provided an odds ratio of 1.67 (95% CI = 0.98-2.87). Other studies, such as the Iowa Women's Health Study, also observed an increase in the odds ratio for dietary calcium after adjustment for potentially confounding variables.[34]

Significant inverse associations with consumption of fermented dairy products have been found in two American case-control studies.[14,15] Besides the choice of control group, the apparent contradiction between our study and or experimental studies might be explained by differences in bacterial strains in local American products and differences in experimentally used strains. In the Netherlands *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, *S. tremor* and *S. lactis* are the main species used for the fermentation process of yogurt. These strains are thought to be less resistant to acidic conditions in the stomach while their survival might be essential for a favourable effect in the colon.[35]

Previous epidemiological case-control studies on the role of calcium in colorectal carcinogenesis have yielded conflicting results. Although some case-control and prospective studies suggest a protective role of calcium and dairy products against the risk of colorectal cancer,[25,36,37] more recent retrospective and prospective studies do not support a protective effect for dietary calcium[18,23] or only support an inverse relation with colon cancer risk in women.[24]

Although the calculations of Garland and colleagues[38] and Newmark et al.[1] suggest a considerable reduction of risk with a calcium intake of more than 1,200 or 1,500 mg per day, respectively, dietary intake of calcium in the general population might not be high enough to observe a decrease in risk. In a recent prospective cohort of women in Iowa, no protective effect of dietary calcium was observed (RR = 0.95), while an inverse association was found for calcium from supplements after adjustment for several covariates (RR = 0.66; 95% CI = 0.43-1.02; p for trend = 0.32).[23]

Human experimental studies suggest a decrease in cell proliferation with supplemental calcium intake of 1,000 to 2,000 mg per day.[10,39-42] However, even with these high doses, some recent experimental studies fail to observe an effect of calcium on overall cell proliferation.[33,43-45] If calcium exerts an effect on cell proliferation it may be particularly important in the early stages of tumor development: i.e. the formation and growth of colorectal adenomas. However, in the Health Professionals Follow-up Study and the Nurses' Health Study, dietary and supplemental calcium intake was not associated with colorectal adenoma risk.[32]

We observed a difference in risk between men and women, which is supported by a recent case-control study in Washington state:[24] a lower risk of colon cancer was found with higher intakes of calcium among women only. Since women are traditionally more involved in the preparation of the main dish, they might recall their habits more accurately than men. To attenuate recall differences between genders in our study, spouses were asked to be present at the time of the home interview. If recall of diet cannot explain gender differences in the associations found, biological mechanisms might differ between men and women. Besides differences in gut metabolism between men and women, the results of a recent controlled experimental trial suggest gender differences in colonic bacterial population and general colonic milieu.[46] It is plausible that differences in bile acid metabolism exist between men and women.[47] As far as fermented dairy products are concerned, certain strains of lactic acid bacteria might colonize the female colon more easily than the male colon.

In summary, this case-control study does not provide support for a protective role of commercially available fermented dairy products or dietary calcium against colon cancer risk. The lack of an inverse association can probably not be explained by information or selection bias. Nevertheless, it would be worthwhile to study this hypothesis prospectively in a population with a similar wide range in intake. It is suggested that experimental studies focusing on differences between male and female bile acid metabolism and large bowel flora might explain the observed differences between men and women.

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Chapter 6

Reproductive and hormonal factors in male and female colon cancer

Abstract

We analysed data from a case-control study in the Netherlands in order to investigate whether reproductive events and hormonal factors are similarly related to colon cancer risk in men and women after adjustment for dietary factors. In total, 232 colon cancer cases (102 women, 130 men) and 259 controls (123 women, 136 men) were interviewed about life-style, medical conditions, and usual dietary patterns, using a structured dietary history technique.

In women, age at first childbirth was positively associated with colon cancer risk (odds ratio (OR) age $\geq$ 26 vs. < 26 = 1.7, 95% confidence interval (CI) = 0.9-3.3). Women with three or more children were at reduced risk compared with women with one or two children (OR = 0.6, 95% CI = 0.3-1.1). When women had had their first child after the age of 26 years, parity was observed to be important (for 1-2 children vs. $\geq$ 3 children OR = 2.8, 95% CI = 1.1-7.0). For men, opposite but non-significant associations were found. Adjustment for dietary patterns and other risk factors did not change the estimates markedly.

Of the hormonal factors, late age at menarche decreased risk (OR = 0.5, 95% CI = 0.3-0.9) while late age at natural menopause slightly increased risk.

Our study provides additional support for the role of reproductive status in the aetiology of female colon cancer independently of dietary factors.

Introduction

Based on gender differences in colon cancer incidence and tumor site distribution, it has been postulated that hormonal and reproductive factors play a role in the aetiology of this cancer.[1] These gender differences in risk, however, might also be attributed to reproductive factors influencing behavioural factors, such as diet and physical activity.[2]

McMichael and Potter[1] proposed the 'hormonal hypothesis', postulating that endogenous oestrogen affects hepatic cholesterol metabolism and increases bile acid production. High concentrations of secondary bile acids in the colonic lumen are thought to enhance colon carcinogenesis.[3] Pregnancy induces a long-term shift in oestrogen profile and reduces bile acid formation. Progestins and exogenous oestrogen at high doses also decrease bile acid synthesis.[1] Under this hypothesis, reproductive events could be associated with colon cancer risk among women but not among men.

In order to evaluate this hypothesis, many analytical epidemiological studies have been conducted and reviewed recently.[2,4,5] Only four of these studied the association with reproductive factors in both sexes separately.[6-9] In two of these studies, the number of children a subject had appeared to decrease colorectal cancer risk in men as well as in women,[7,8] which suggests that life-style factors associated with parity might be important, such as higher physical activity in larger families and altered dietary patterns.

In most studies that investigated reproductive factors, total energy intake and the intake of nutrients relevant to colon cancer were not adjusted for adequately. We studied the relationship between reproductive factors in women and men and hormonal factors in women, taking into account dietary factors and other risk factors of colon cancer.

Subjects and methods

A case-control study of colon cancer was conducted in the Netherlands between 1989 and 1993. Men and women of West European origin, up to the age of 75 at diagnosis, who resided in the central or eastern part of the Netherlands, spoke Dutch and did not have a history of cancer, ulcerative colitis or Crohn's disease were eligible for this study.

Cases
Cases were defined as newly diagnosed and histologically confirmed patients with

adenocarcinomas of the colon (ICD 153). They were recruited as soon as possible after diagnosis by their medical specialists in 14 regional hospitals. The cancer registries of the central and eastern parts of the Netherlands were used as a check on completeness and provided additional clinical and pathological information. For 28 participants, the cancer registry defined the location of the tumor as rectosigmoid while according to the surgeon the tumor was located in the colon. Analyses were conducted including and excluding these cases.

Of all eligible cases diagnosed in the 14 cooperating hospitals, 47% were invited by the surgeon to participate. Non-invitation was mainly due to administrative and organizational reasons. Comparison with cancer registry data showed that cases invited did not differ importantly from those not invited with respect to age, sex, urbanization grade and site distribution. However, uninvited cases did have a worse prognosis according to Dukes' status and underwent radiotherapy and chemotherapy more frequently. Of those invited, 60% agreed to be interviewed. Except for Dukes' status, the distributions of age, gender, tumor site and therapies of the interviewed cases was comparable to the distribution among all eligible cases. A short questionnaire, which included questions on the most relevant dietary and life style factors, was send to the participants as well as the invited non-participants. All participants and 23% of these non-participants completed this questionnaire and returned it. No important differences were observed between participants and non-participants. Because of the low response among non-participants, these data were not incorporated into full analysis. Although the number of participants would have been larger, it would also have been less precise.

The average interval between diagnosis and interview was three months.

Controls
Frequency matched population controls, matched for age (on 5-year intervals), gender, region and degree of urbanization as the cases, were recruited randomly by the general practitioners of the participating cases. For each case, the general practitioner received a standardized form with three three-letter strings, randomly obtained from the local telephone directory to initiate the search for controls in his or her own database; for each string, the first subject who matched the eligibility criteria was sent a standard letter of invitation, including a reply form to the investigator. Of the 520 controls invited, 57% agreed to participate in the interview. Participation was higher among younger people and those living in urban areas. Dietary and life style patterns did not differ significantly between those who participated in the interview and those who refused to participate but completed the non-response questionnaire (42% of non-participants).

Questionnaire

A total of seven trained dieticians conducted personal interviews in the participant's home. In order to contain comparable information of cases and controls, each interviewer visited a similar number of cases and controls from the same age, gender, region and urbanization grade. The structured interview included questions on sociodemographic indicators (e.g. age, education, occupation, urbanization grade), medical history (e.g. cholecystectomy), family history of colon cancer, reproductive factors (number of children and age at first birth). Female participants were asked about the type and duration of use of oral contraceptives (OC) and use of oestrogen-replacement therapy, ages at menarche and menopause, and type of menopause (natural or surgical). Occupation was used as a measure of socioeconomic status (SES); socioeconomic status of married women (including divorcees and widows), was based on their husband's (or former/late husband's) occupation.

Data on dietary habits were collected, using a structured dietary history questionnaire[10] referring to the previous full year (in cases before symptoms occurred). Interviewing cases and controls was balanced over seasons to account for seasonal fluctuations on food patterns. Intake of carbohydrates, fat, fiber and alcohol were adjusted for energy intake by regression analysis[11] for men and women separately.

Data analysis

Odds ratios (OR) as estimators of relative risks and approximate 95 % confidence intervals (95% CI) were calculated by unconditional maximum likelihood estimation using BMDP software.[12] To control simultaneously for the potential confounding effects of age, urbanization grade, energy intake, energy-adjusted intake of fat, carbohydrates, dietary fiber and vitamin C, socioeconomic level, cholecystectomy, and family history of colorectal cancer, multiple logistic regression models were used.

Because of the low numbers of childless cases (7 women and 11 men), subjects with one or two children were used as reference. Mean age at birth of first birth (women 26 years; men 28 years) was used to divide into the low and high age at first birth categories.

Exclusion of potential rectosigmoid cases did not change the results appreciably (data not shown).

Results

Table 6.1 gives the distribution of risk factors among cases and controls, for men

and women separately. Cholecystectomy and positive family history were more
prevalent among cases, but due to the stratification by sex, this was of borderline
significance in either men or women (0.05 < p < 0.1). No other statistic significant
differences were observed.

Table 6.1   Mean and standard deviation (or standard error[†]) of some characteristics in 232
            people with colon cancer cases and 259 controls subjects.

	women		men	
	cases n = 102	controls n = 123	cases n = 130	controls n = 136
Age (years)	61.3 ± 10.4	61.2 ± 10.5	62.7 ± 10.1	62.3 ± 9.4
Urbanization grade (% urban)	45.1 ± 4.9	50.4 ± 4.5	51.5 ± 4.3	55.1 ± 4.4
Reproductive factors				
Number of children	2.7 ± 1.6	2.7 ± 1.8	2.8 ± 1.7	2.6 ± 1.8
Age at first birth among				
parous (years)	26.3 ± 4.5	25.9 ± 3.9	27.8 ± 4.8	28.7 ± 4.4
Hormone-related factors				
Age at menarche (years)	13.5 ± 1.8	13.7 ± 1.5	-	-
Age at menopause (years)	48.9 ± 5.2	48.5 ± 5.2	-	-
OC use (%)	47.9 ± 5.1	47.9 ± 4.5	-	-
Other factors				
Cholecystectomy (%)	16.2 ± 3.7	8.1 ± 2.5*	6.2 ± 2.1	3.8 ± 1.7
Family history of				
colon cancer (%)	21.1 ± 4.2	13.6 ± 3.2	17.5 ± 3.9	9.5 ± 2.6*
Body mass index (kg/m²)	25.6 ± 4.9	25.6 ± 4.3	26.2 ± 3.7	26.3 ± 3.3
Defecation frequency				
(% more than once/day)	21.0 ± 4.1	14.6 ± 3.2	30.7 ± 4.1	36.0 ± 4.1
Socioeconomic level				
(% blue collar)[†]	50.0 ± 5.1	42.9 ± 4.5	44.9 ± 4.4	38.2 ± 4.2

* p = 0.07.
[†] Instead of SD, SE = $\sqrt{(p(1-p)/n)}$ is given for proportions.

With respect to reproductive- and hormonal-related factors, female controls,
compared with cases, tended to have lower age at first birth. Although differences
were not significant, defecation frequency was higher among female cases, but
lower among male cases than in controls. A higher proportion of female and male
cases seemed to have a lower socioeconomic level.

    With respect to dietary variables, higher energy intake was found among both
female and male cases (Table 6.2). After adjustment for energy intake, cases tended
to consume more fat and less carbohydrates, for men these associations did not
reach statistical significance. Also, energy-adjusted dietary fiber intake and vitamin

C intake were slightly higher in male cases, and significantly higher in female cases. The proportion of alcohol consumers was similar, but among those who consume alcohol mean alcohol intake tended to be higher in cases for both men and women.

Table 6.2   Average daily intake (mean ± SD or SEM[$]) of energy and nutrients[†] in 232 people with colon cancer and 259 controls subjects.

	women		men	
	cases n = 102	controls n = 123	cases n = 130	controls n = 136
Energy (kJ)	8,785 ± 256	7,841 ± 2,129**	11,727 ± 312	10,737 ± 2,711**
Total fat (g)	86.0 ± 14.0	82.5 ± 14.4*	117.7 ± 23.0	116.9 ± 20.8
Total protein (g)	74.6 ± 12.8	74.9 ± 11.9	93.3 ± 14.7	91.5 ± 12.9
Total carbohydrates (g)	213.5 ± 42.0	224.6 ± 36.7**	272.1 ± 56.1	281.1 ± 49.2
Dietary fiber (g)	24.2 ± 6.6	26.6 ± 6.7**	30.3 ± 8.9	31.1 ± 9.0
Vitamin C (mg)	108.5 ± 51.5	129.6 ± 58.6**	107.6 ± 53.4	112.3 ± 48.2
Alcohol				
% Drinkers[$]	72.5 ± 4.4	68.3 ± 4.2	86.9 ± 3.0	90.5 ± 2.5
Alcohol (g)[§]	11.5 ± 15.3	9.4 ± 10.4	27.2 ± 29.0	22.9 ± 20.6

* $p < 0.1$.
** $p < 0.05$.
[†]   Energy adjusted by regression for men and women separately.
[$]   SE $\sqrt{(p(1-p)/n)}$.
[§]   Among alcohol consumers.

Table 6.3 presents ORs for reproductive factors, unadjusted and adjusted for medical and dietary factors. After adjustment ORs became stronger, but this did not change the results dramatically. For women, risk was inversely associated with number of children (OR = 0.56) and positively associated with age at first birth (OR = 1.69). For men, associations with both variables were in the opposite direction.

When both reproductive variables were studied simultaneously, increased risk was essentially limited to women with few children, who had their first child after the age of 26 years (OR = 2.79) (Table 6.4). Additional adjustment for use of oral contraceptives attenuated the OR slightly (OR age at first birth ≥ 26, number of children 1-2 = 2.48, 95% CI = 0.96-6.44). For men, a decreased risk was observed in the corresponding exposure category.

Table 6.3 Reproductive factors among men and women and colon cancer risk.

	Women				Men			
			Odds Ratios (95% CI)				Odds Ratios (95% CI)	
Variables	Cases* (n)	Controls (n)	Age-adjusted	Multivariate$	Cases* (n)	Controls (n)	Age-adjusted	Multivariate$
Number of children								
1-2	44	41	1.00[†]	1.00[†]	46	59	1.00[†]	1.00[†]
≥ 3	49	64	0.68 (0.37-1.22)	0.56 (0.28-1.11)	72	59	1.57 (0.93-2.64)	1.49 (0.84-2.64)
Age at first birth(years)								
< 26[§]	38	55	1.00[†]	1.00[†]	60	51	1.00[†]	1.00[†]
≥ 26	54	50	1.56 (0.88-2.75)	1.69 (0.87-3.27)	58	67	0.73 (0.44-1.24)	0.69 (0.39-1.23)

Adjusted for age, urbanization grade, energy intake, energy-adjusted intake of fat, carbohydrates, dietary fiber and vitamin C, cholecystectomy, family history of colon cancer and socio-economic level.
Reference category.
For men < 28 vs. ≥ 28.
Missing data on both variables in 2 cases, for 1 case age at first birth was unknown.
Missing data on both variables in 1 case.

Hormonal factors (for women) are presented in Table 6.5. Risk was significantly lower for a menarche at age 14 than for an earlier menarche (OR = 0.51, 95% CI = 0.28-0.94). Women who had a natural menopause after the age of 50 had a slightly increased risk relative to those with an earlier natural menopause. Artificial menopause and use of oral contraceptives were not associated with colon cancer. Additional adjustment for parity did not change the association with use of oral contraceptives importantly (OR = 1.10, 95% CI = 0.49-2.48).

Table 6.4   Number of children in different age categories at first birth and colon cancer risk (232 cases, 259 controls).

	Women Age at first birth (yr)		Men Age at first birth (yr)	
	< 26	≥ 26	< 28	≥ 28
1-2	1.35 (0.50-3.68)	2.79 (1.10-7.03)	0.81 (0.33-2.01)	0.55 (0.27-1.13)
≥ 3	1.00[†]	1.33 (0.55-3.20)	1.00[†]	0.82 (0.38-1.79)

Odds ratios and (in parentheses) 95% confidence intervals adjusted for age, urbanization grade, energy intake, energy-adjusted intake of fat, carbohydrates, dietary fiber and vitamin C, cholecystectomy, family history of colon cancer and socioeconomic level.
[†]   Reference category.

Table 6.5   Hormonal factors among women with colon cancer (n = 102) and control subjects (n = 123).

Variables	Cases* (n)	Controls (n)	OR (95% CI)	
			Age-adjusted	Multivariate[$]
Age at menarche (years)				
≤ 13	53	52	1.00[†]	1.00[†]
≥ 14	44	67	0.65 (0.38-1.11)	0.51 (0.28-0.94)
Menopause				
natural, at < 50 years	18	30	1.00[†]	1.00[†]
natural, at ≥ 50 years	37	36	1.73 (0.81-3.67)	1.38 (0.58-3.31)
artificial	21	26	1.32 (0.58-3.03)	0.95 (0.36-2.47)
Oral contraceptive use				
no	50	63	1.00[†]	1.00[†]
yes	46	58	1.02 (0.53-1.93)	0.97 (0.46-2.03)

[$]   Adjusted for age, urbanization grade, energy intake, energy-adjusted intake of fat, carbohydrate, dietary fiber and vitamin C, cholecystectomy, family history of colon cancer and socioeconomic level.
[†]   Reference category.
*   Strata do not add up to the total because of missing data.

physical activity increases strongly with number of children. Nor can physical activity explain the importance of number of children in group older at the time of their first child's birth.

The lower risk in men at older age with fewer than three children as compared to younger men with three or more children might be explained by socio-economic level, which is related to reproductive as well as other factors. However, adjustment for socioeconomic level using different classification systems did not change the results.

Other indicators of a long-lasting or high gastrointestinal exposure to oestrogens are an early age at menarche, late menopause and use of oral contraceptives. Except for age at menarche, no significant associations were observed. An inverse association between age at menarche and colon cancer risk was observed in another European study;[13] five other studies[4,7,14-16] did not find such an association. Franceschi et al.[4] found a similar (non-significant) protection against colon cancer for premenopausal women, but no difference between natural and artificial menopause, whereas Wu et al.[7] found an increased risk associated with surgical menopause (RR = 1.7, 95% CI = 1.0-2.9). Late age at menopause was found to be weakly protective in some[4,7,14] but not in others.[13,15,16] Use of oral contraceptives showed no relation with colon cancer risk in our study and other studies.[7,14,15,17]

The observed differences in associations between reproductive events and colon cancer risk for men and women support a role of endogenous female sex hormones in the aetiology of colon cancer independently of dietary factors. The mechanisms of these hormonal influences need to be explored further.

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Abstract

To examine the relationship between colon cancer and vegetable or animal products, we analyzed data from a Dutch case-control study. Dietary patterns were assessed for 232 colon cancer cases and 259 population controls.

In multivariate analyses, the consumption of vegetables was significantly associated with reduced colon cancer risk (Odds Ratio highest vs. lowest quartile of consumption (OR) = 0.4, 95% Confidence Interval (CI) = 0.2-0.7, p-trend = 0.0004). Consumption of fresh red meat was positively associated with risk in women (OR = 2.4, 95% CI = 1.0-5.7, p-trend = 0.04), especially for those with a relatively low consumption of vegetables and fruits (OR = 3.1). For men, no association with fresh red meat consumption was found (OR = 0.9). No clear associations were found for other products of vegetable or animal origin.

The results of this Dutch case-control study support the preventive potential of a high vegetable-diet in colon cancer risk. This study suggests this may be important for women consuming a diet high in red meat.

Introduction

Colon cancer is one of the most prominent types of cancer in the Netherlands, as in many other Westernized countries. In Dutch men, colon cancer incidence ranks third (7%), behind lung cancer (24%) and cancer of the prostate (14%). For Dutch women, cancer of the colon is the second most common type (10%), after breast cancer (31%).[1]

Dietary patterns are considered to contribute importantly to the etiology of colon cancer. Research focused primarily on two nutrient hypotheses: dietary fiber is protective, while fat intake increases the risk of colon cancer. Results have been both affirmative and contradictory for nutrients and food groups.

Nowadays, the dietary fiber hypothesis is mostly interpreted as an effect of vegetables and/or fruits, with a variety of nutrients and bioactive substances relevant to the prevention of cancer of the colon as well as many other cancer sites. Although several epidemiological studies addressed the association between colon cancer risk and the consumption of vegetables and fruits as reviewed by Steinmetz and Potter[2] and Block et al.,[3] none of these studies were conducted in the Netherlands. Most previous studies in Westernized countries support a protective effect for vegetables: about two-third of the case-control studies observed an inverse association with colon cancer risk for at least one vegetable and/or fruit food group,[4-18] while others reported no association or a positive one.[19-23]

Red meat consumption is one of the major sources of saturated fat in a westernized diet. In contrast to the consumption of vegetables, red meat consumption as related to colon cancer risk has been studied in the Netherlands Cohort Study on diet and cancer which showed no important association between red meat consumption and colon cancer risk.[24] Although supported by other studies,[5,16,17,21,25] these findings contradict results of several studies showing a significant positive association with red meat consumption.[4,11,13,15,18,23,26] Although differences in dietary methodology and subject recruitment might partially account for such inconsistencies, it may be that meat consumption is not important in itself, but only relative to protective factors provided by vegetables and fruits.[24] Recently, Steinmetz and Potter[27] studied the ratio of the consumption of red meat to that of vegetables and fruits. Although not significantly so, this ratio was higher for cases than for controls both in men and women.[27] A similar approach was used in the Nurses' Health Study for the ratio of red meat to fish and chicken.[26]

Moreover, there are many indications that the association between diet and colon cancer might be different for men and women. However, previous studies reporting gender-stratified analyses have produced inconsistent findings.[6,9,12,18,27]

In a case-control study, we examined the role of vegetables, fruits, red meat and their ratio as related to colon cancer in Dutch men and women. In addition, we explored associations and potential gender interactions for other food groups of vegetable and animal origin, which have been linked to the risk of colon cancer such as potatoes,[28] dairy products,[29] eggs,[27] fish and poultry.[26] Since the numerous components of foods might, through interaction, produce effects which are different from those of the exposure to a single nutrient and interpretation in terms of preventive potentials is easier, we focused especially on the consumption of food groups.

Subjects and methods

Subjects

The population based case-control study was conducted in the Netherlands between 1989 and 1993. Cases were defined as newly diagnosed male and female patients with adenocarcinoma of the colon (ICD-O 153). They were recruited from regional hospitals from east and central Netherlands. The cancer registries of these areas were used as a check on completeness and provided additional clinical and pathological information. Cases were Western-European with the Dutch nationality, who were at time of diagnosis up to 75 years old and did not have previous cancer, polyposis coli, ulcerative colitis or Crohn's disease. For 28 cases, the cancer registry defined the location of the tumor as rectosigmoid in contrast with the surgeon who located the tumor in the colon. Analyses were conducted including as well as excluding these cases.

Of all eligible cases diagnosed in the cooperating hospitals, 47% was invited to participate. Non-invitation was mainly due to technical and administrative reasons. Cases invited did not differ importantly from those not invited in age, gender, urbanization-level and site distribution. Cases who were not invited did have a worse prognosis according to Dukes' status and more frequently underwent radiotherapy and chemotherapy. Of those invited, 60% agreed to be interviewed. Except for Dukes' status, the distributions of age, gender, tumor site and therapies of the interviewed cases was comparable to the distribution among all eligible cases. In order to explore potential selection bias, participants and non-participants were asked to fill out a short questionnaire on life-style habits and the consumption frequency of dairy products, bread and meat. All participating cases and 23% of the non-participating cases filled out the questionnaire and returned it. Cases participating in the interview did not differ significantly in the consumption of food groups of major interest from those non-participants who filled out the short ques-

tionnaire.

The population controls were recruited randomly by general practitioners of participating cases, using the same eligibility criteria as for the cases. They were frequency matched on age, sex and degree of urbanization to the cases. Of the 520 controls invited, 57% agreed to participate in the interview. Participation was higher among younger people and those living in urban areas. Meat intake, other dietary and life-style patterns did not differ importantly between those who participated in the interview and those who refused to participate but completed the non-response questionnaire (42% of the non-participants).

Data collection
Cases and controls were interviewed on dietary habits and life-style factors in their own homes by qualified dietitians. For cases, the interval between diagnosis and interview was three to six months. Each dietitian interviewed a similar number of cases and controls in a geographical area. The dietary part of the questionnaire requested information on the frequency of consumption during 1 year prior to interview (for cases before diagnosis or complaints) and consisted of a detailed structured dietary history questionnaire, covering the complete dietary pattern. The frequency of consumption per month, number of months in which the item was consumed, the number of portions per consumption and the size of the portions of 289 food items from 19 food groups were asked for. To be able to estimate portion sizes, frequently used household utensils and cups were weighed.

To minimize errors, the dietary history questionnaire was entered into the computer using a specially designed computer program which included range-checks and cross-checks. Checks on the internal consistency of the frequency of consumption of various meal components with the total meal pattern were conducted. Frequencies of individual items were corrected automatically in order to agree with the frequencies of the total food group. Average daily intake of nutrients was calculated using the Dutch National Food Table.[30] Intake of nutrients was adjusted for energy intake by regression analysis for men and women separately.[31]

In addition to diet, subjects were asked about socio-demographic characteristics (e.g. age, urbanization-level, education and occupation), menstrual and reproductive history for women, positive family history of colon cancer, personal medical history, smoking habits and past use of medications. To address stability of food habits, changes in habits as a result of gastro-intestinal complaints were asked for.

Data analysis
Differences in demographic, predisposing, dietary and life-style factors were compa-
red between cases and controls. The individual food items in the questionnaire were

categorized into major food groups (e.g. vegetables, fruit, red meat (including fresh red meat) and poultry) which intake was expressed in grams per day. Ratios were calculated for red meat to poultry and fish and for red meat to vegetables and fruits. Calculating ratios, non-users were excluded from the analyses (4 non-users of red meat and 26 non-users of fish or poultry; each participant consumed vegetables or fruits). Including non-users did not change the results importantly (data not shown).

Nutrient intake, food group consumption and the ratios were divided into quartiles according to the distribution in the control group. Odds ratios (OR) for colon cancer as estimates of relative risks, together with their approximate 95% confidence intervals (95% CI), were calculated by maximum likelihood estimation using BMDP software, taking the lowest quartile of exposure as the reference. To account simultaneously for the potential confounding effect of age, gender, urbanization-level, energy intake, vitamin C intake (only with nutrient intake), family history of colon cancer, cholecystectomy, socio-economic status, body mass index, smoking habits and alcohol intake, multiple logistic regression models were used. Decisions on which covariates to include in the final models were based on 1) evidence from epidemiological literature, 2) biological plausibility, 3) whether the regression coefficient of the primary independent variable changed ≥ 10% after addition of the potentially confounding variable and 4) whether the covariate entered the model at the 0.10 level of significance.

Chi-square tests for trend were conducted using the median of each quartile as a continuous variable. Chi-square tests for gender interaction were conducted.

Additionally, odds ratios of those food groups for which an interaction with gender was observed were calculated for men and women separately, with the quartiles based on the distribution of the intake of male and female controls, respectively.

Results

Mean age was similar among cases and controls (Table 7.1). The case group included more men, less current smokers, less people living in urban areas and a higher proportion of people with lower socio-economic status.

Family history of colon cancer and cholecystectomy was more frequent among cases. The intake of energy, energy-adjusted intake of total fat and total protein was higher among cases, while the energy-adjusted intake of total carbohydrates, dietary fiber, vitamin C and alcohol was less. Total supplement use and use of specific calcium and vitamin D supplements tended to be lower in cases. Cases and controls did not differ in body mass index.

Table 7.1 Mean and standard deviation of some characteristics in 232 colon cancer cases and 259 controls.

Characteristics	Cases (n = 232) mean ± SD	Controls (n = 259) mean ± SD
Demographic factors		
Age (years)	62 ± 10	62 ± 10
Sex (%male)[#]	56 ± 3	53 ± 3
Urbanization-level (% urban)[#]	48 ± 3	53 ± 5
Socio economic status (% blue collar)[#]	46 ± 9	40 ± 3
Predisposing factors		
Family history of colorectal		
cancer (%)[#]	18 ± 3	11 ± 2
Cholecystectomy (%)[#]	10 ± 7	5 ± 6
Dietary factors[¶]		
Total energy intake (kJ)	10,433 ± 3,234	9,362 ± 2,844[‡]
Total fat (g)	104 ± 25	101 ± 25
Total carbohydrates (g)	246 ± 58	254 ± 52
Total protein (g)	85 ± 17	84 ± 15
Dietary fiber (g)	28 ± 9	29 ± 8
Vitamin C (g)	105 ± 53	118 ± 54[‡]
Life-style factors		
Body mass index (kg/m^2)	26 ± 4	26 ± 4
Current smokers (%)[#]	29 ± 3	33 ± 3
Alcohol (g)[¶]	11 ± 25	14 ± 18
Supplement use		
Total (%)[#]	34 ± 3	37 ± 3
Calcium and or vitamin D (%)[#]	11 ± 2	19 ± 2[‡]

[#] SE = $\sqrt{(p(1-p)/n)}$.
[¶] adjusted for total energy intake by regression analysis, separately for men and women.
[‡] $p < 0.05$.

Table 7.2a presents odds ratios of colon cancer for quartiles of intakes of several food groups. Considering food groups of vegetable origin, a significant inverse association with vegetable consumption was found after adjustment for total energy and for variables for which frequency matching occurred. For other food groups of vegetable origin no significant associations were found: OR ranged from 0.67 for potatoes to 1.16 for cereal products (Table 7.2a). For the consumption of legumes no important associations were found as well (OR = 1.08; 95% CI = 0.67-1.76). However, in this population legumes were consumed too infrequent to draw firm conclusions.

Table 7.2b.   Total energy intake and the intake of energy-adjusted nutrients as related the risk of colon cancer (both genders combined).

Variable	Q1	Q2	Q3	Q4	χ^2 trend (p-value)	χ^2 gender inter-actions (p-value)
Energy intake (Kj)	< 7,476	7,476-8,857	8,858-11,097	> 11,097		
cases/controls	39/65	46/65	57/65	90/64		
OR(95% CI)[§]	1.00	1.36 (0.76-2.41)	1.74 (0.98-3.08)	3.17 (1.70-5.91)		0.48 (0.49)
OR(95% CI)[¶]	1.00	1.36 (0.76-2.45)	1.82 (1.01-3.30)	3.47 (1.83-6.58)	17.57 (0.0000)	
Vitamin C[+]	< 79.0	79.0-110.8	110.8-146.6	> 146.6		
cases/controls	73/65	73/65	49/65	37/64		
OR(95% CI)[§]	1.00	1.04 (0.64-1.69)	0.66 (0.40-1.11)	0.51 (0.30-0.88)		2.53 (0.11)
OR(95% CI)[¶]	1.00	1.07 (0.65-1.76)	0.70 (0.42-1.19)	0.53 (0.31-0.92)	6.91 (0.009)	
β-Carotene[+]	< 1.8	1.8-2.4	2.4-3.4	> 3.4		
cases/controls	81/63	50/67	53/65	48/64		
OR(95% CI)[§]	1.00	0.64 (0.39-1.07)	0.69 (0.42-1.15)	0.60 (0.36-1.00)		0.32 (0.57)
OR(95% CI)[¶]	1.00	0.64 (0.38-1.06)	0.70 (0.42-1.16)	0.61 (0.36-1.03)	2.91 (0.09)	
Dietary fiber[+]	< 23.1	23.1-27.8	27.8-33.4	> 33.4		
cases/controls	82/65	49/65	49/65	52/64		
OR(95% CI)[§]	1.00	0.58 (0.35-0.97)	0.58 (0.34-0.97)	0.56 (0.33-0.95)		3.54 (0.06)
OR(95% CI)[¶]	1.00	0.57 (0.34-0.96)	0.63 (0.37-1.06)	0.62 (0.36-1.08)	2.47 (0.12)	

Animal protein[†]	< 48.2	48.2-56.5	56.5-65.4	> 65.4		
cases/controls	54/65	45/65	66/65	67/64		
OR(95% CI)[§]	1.00	0.87 (0.50-1.50)	1.40 (0.82-2.40)	1.25 (0.72-2.17)		0.09 (0.77)
OR(95% CI)[¶]	1.00	0.85 (0.48-1.49)	1.39 (0.80-2.41)	1.32 (0.75-2.33)	1.88 (0.17)	
Saturated fat[†]	< 33.4	33.4-40.7	40.7-49.5	> 49.5		
cases/controls	42/64	64/66	64/65	62/64		
OR(95% CI)[§]	1.00	1.71 (0.99-2.95)	1.80 (0.96-3.38)	1.63 (0.82-3.23)		0.44 (0.51)
OR(95% CI)[¶]	1.00	1.53 (0.87-2.69)	1.55 (0.80-3.01)	1.37 (0.66-2.85)	0.08 (0.78)	
Cholesterol[†]	< 226.2	226.2-286.0	286.0-347.2	> 347.2		
cases/controls	45/65	59/65	60/65	68/64		
OR(95% CI)[§]	1.00	1.58 (0.92-2.73)	1.71 (0.97-3.03)	1.73 (0.95-3.14)		0.53 (0.47)
OR(95% CI)[¶]	1.00	1.43 (0.82-2.50)	1.54 (0.86-2.78)	1.48 (0.80-2.76)	1.21 (0.27)	

[§] adjusted for age, gender, urbanization-level and total energy intake.
[¶] adjusted for age, gender, urbanization-level, total energy intake, alcohol intake, vitamin C intake (not for β-carotene and dietary fiber), cholecystectomy, family history of colorectal cancer.
[†] mg per day, adjusted for total energy intake by regression, separate for men and women.

tumors would have participated in the study, we might have found stronger invers
associations for vegetables. Meat consumption was not related to Dukes' status in
this study. Participating and non-participating cases did not differ in mea
consumption either. Since recall of dietary habits can be influenced by a mor
severe prognosis or medical treatment influencing appetite, it may be an advantage
to have cases with better prognosis participating in the study. Age, gender and
urbanization-level from interviewed cases did not significantly differ from those
eligible.

In the Netherlands, a vegetable rich diet is related to a healthy life-style in
general and a higher socio-economic status. If selection of more health-consciou
controls occurred, it might explain the results of this study. However, ou
population-based control group was comparable to the general population according
to the distribution of socio-economic status.

In order to minimize information, cases and controls were interviewed in th
same season using a structured dietary history questionnaire. Interviewers wer
trained by the same experienced dietician, who intensively monitored their inter
viewing and coding. Each interviewer assessed dietary habits of an similar numbe
of cases and controls. No differences between interviewers were found.

Recall bias might influence the results especially when cases, as a result of thei
disease, report their usual intake before symptoms occurred or the disease wa
diagnosed differently from the controls. Differential overreporting could occur i
some cases remember consuming more foods in general by concentrating mor
intensively, which is supported by the observed positive association with tota
energy intake. However, adjustment for total energy intake could not explain ou
findings. Social desirability of eating healthy foods, such as vegetables and fruits
might influence recall as well. Since the questionnaire was structured, and th
complete dietary pattern and several life-style habits were inquired about, it may b
assumed that participants were unaware of the hypothesis tested. Finally, changes o
habits as a result of gastro-intestinal distress might also be an important reason fo
recall bias. However, excluding those who indicated to have changed their dietar
habits because of complaints did not change the results importantly.

In conclusion, selection and information bias do not seem to have distorted th
internal validity of the study seriously.

Food groups of vegetable origin
With respect to <u>vegetable</u> consumption, our study confirms the results of th
majority of other case-control studies on colon cancer which observed invers
associations with at least one vegetable group.[2] However, most studies reported non

vegetables. Differences between studies might be a result of different consumption patterns among the populations studied. The consumption of vegetables in our study population is relatively high as compared to other studies, and is also high when compared with the intake among the general Dutch population, which is assessed using a 2-day record.[32] Although checks have been included in the data entry program to compare the reported total frequency of vegetable consumption with the reported consumption of individual vegetables, this might just be a consequence of the lengthy dietary history method used.

Taking power considerations into account and the fact that the examination of a large number of food items might increase the possibility of chance findings and therefore introduce over-interpretation, we explored the associations with food groups and not of individual food items. Previous studies observed inverse associations between colon cancer risk and green leafy vegetables[8] and cruciferous vegetables.[13,35] The majority of vegetables consumed in this population were cruciferous vegetables and green leafy vegetables.

Vegetable consumption was more strongly related to risk than <u>fruits</u>. This is not in line with the case-control study of Steinmetz and Potter[18] conducted in Australia. In our study an inverse association with fruit consumption was only observed among women, which is in line with the results for fruits in other[12,18] but not all[9] case-control studies.

<u>Potatoes</u> are an important source of vitamin C and polysaccharides. We observed an inverse association with potato consumption. This is in line with a few other case-control studies,[5,21] while in some studies an increased risk with potato consumption was found.[11,13,18] Nevertheless, as potatoes are often consumed as part of the main meal, the observed inverse association might be an underestimation of the true association, because of the associated consumption of meat or the use of fat-laden condiments.

Food groups of animal origin
Except for a positive association with red meat consumption in women, for food groups of animal origin, only non-significant associations were found. None of the quintile specific OR's nor trends were statistically significant.

A higher consumption of <u>fish or poultry</u> did not importantly decrease colon cancer risk as suggested by other studies.[26,27] Furthermore, the ratio of red meat to fish and poultry was not associated with risk, which is in line with the findings of an Australian case-control study,[27] but not with the findings in the Nurses' Health Study.[26]

Consumption of <u>dairy products</u> was weakly positively, but non-significantly related to risk in this population. The results of epidemiological studies have been

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Abstract

Experimental studies suggest that an increased consumption of fermented dairy products and calcium might decrease the risk of colorectal cancer.

The association between fermented dairy products, dietary calcium and colorectal cancer risk was investigated in a population with a wide variation in intake of dairy products. The Netherlands Cohort Study on diet and cancer (NLCS) started in 1986 when 120,852 Dutch men and women, aged 55-69 years, filled out a questionnaire on dietary patterns and life-style. The present analysis is based on 3.3 years of follow-up and included 215 incident cases of colon cancer and 111 incident cases of rectal cancer, excluding cases diagnosed in the first year of follow-up.

After adjustment for potential confounding variables, colorectal cancer risk was weakly inversely associated with the consumption of fermented milk (Relative Rates (RR) in the highest category of intake compared to non-users = 0.89, 95% Confidence Intervals (CI) = 0.60-1.33), unfermented milk (RR = 0.86, 95% CI = 0.57-1.29), and cheese (RR = 0.88, 95% CI = 0.59-1.33). However, category-specific relative rates, and tests for trends were not statistically significant. For fermented milk, the inverse association was limited to colon cancer (RR = 0.70, 95% CI = 0.43-1.15, trend p = 0.33).

In crude and multivariate models, total dietary calcium intake (highest vs. lowest quintile RR = 0.92, 95% CI = 0.64-1.34) and calcium from fermented dairy products (RR = 1.14, 95% CI = 0.77-1.68) were not significantly associated with colorectal cancer risk. Calcium from unfermented dairy products was inversely associated with rectal cancer risk (RR = 0.55, 95% CI = 0.30-1.04, trend p = 0.03).

After 3.3 years of follow-up, these data are not consistent with a substantially decreased risk of colorectal cancer with increased intake of fermented dairy products and dietary calcium.

Introduction

Colorectal cancer is one of the major types of cancer in Western society. In the Netherlands, yearly about 48 new cases of colorectal cancer (30 colon, 18 rectum) are diagnosed per 100,000 men and 49 cases per 100,000 women (35 colon, 14 rectum). Colorectal cancer is the third most common cancer in Dutch men and the second most common cancer in Dutch women.[1] As its incidence is still rising, treatment is difficult, and mortality remains high, emphasis should be placed on preventive measures. Besides dietary recommendations against cancer in general, such as a higher consumption of vegetables and fruits,[2] other relatively simple and feasible dietary changes might add to the prevention of colorectal cancer.

The possible protective role of fermented dairy products such as yogurt, buttermilk and cheese, originally proposed by Metchnikoff[3] in the beginning of this century, has regained attention in the past decade. Establishment of certain lactobacilli in the gastro-intestinal tract could contribute to the stabilization of the colonic flora, and exert beneficial effects on the host. In recent experiments, the activity of carcinogen-producing faecal enzymes was decreased in subjects supplemented with *Lactobacillus* cultures[4,5] and in vitro studies with human lymphocytes suggest that lactobacilli stimulate the immune system locally, as well as systemically.[6,7]

In addition to these mechanisms mediated by microorganisms, fermented dairy products contribute substantially to our daily calcium intake. In the Netherlands, about 70% of calcium intake is of dairy origin, half of which is from fermented dairy products. It is hypothesized that calcium[8] or, more recently, that calcium phosphate[9] binds free fatty acids and bile acids, thereby reducing the effective toxic dose to the colonic epithelial cells and their subsequent proliferative response. Calcium might also directly influence cell proliferation by inducing cell differentiation.[10]

Although several epidemiological studies have investigated the relationship between dairy product consumption, calcium intake and colorectal cancer (e.g.[11-22]) or colorectal polyps (e.g.[23]), only few addressed the association with fermented dairy products.[14,16,17,18,20,23] In two population-based case-control studies on colon cancer an inverse association was observed for yogurt (Odds Ratio (OR) = 0.83 for 10 servings per month, 95% Confidence Intervals (CI) = 0.70-0.98)[20] and cultured milk consumption (OR = 0.65, 95% CI = 0.41-1.01),[16] adjusted for potential confounding variables. In two companion American prospective studies, the 1980-1988 follow-up of the Nurses' Health Study (NHS: 121,700 women) and the 1986-1990 follow-up of the Health Professionals Follow-Up Study (HPFS: 51,529 men),

a non-significant inverse association with yogurt consumption and colorectal polyps was found (RR for more than 4 times a week 1 cup vs. almost never NHS = 0.60 95% CI = 0.30-1.18; HPFS = 0.66, 95% CI = 0.28-1.46), which diminished in the HPFS after adjustment for covariates (RR = 0.89).[23]

Although cheese consumption contributes significantly to saturated fat intake, in several case-control studies[14,16,18] and one prospective study[17] the consumption of this product was not strongly associated with colorectal cancer risk (Relative Rates (RR) 0.9, 0.7, 1.1 and 1.1 respectively).

The studies mentioned above have mainly been conducted in countries in which the consumption of yogurt or cultured milk is not integrated in traditional food habits. In countries in which the consumption of fermented milk is high and heterogeneous, the comparison of risks between high and low consumption categories would be most powerful. The Dutch have a longstanding tradition of fermented dairy product consumption (e.g. per capita annual consumption of yogurt in the Netherlands is 18.9 kg vs. 2.1 kg in the United States).[24] In two case-control studies conducted in the Netherlands inverse associations between breast cancer[25] and cancer of the exocrine pancreas[26] on the one hand and fermented dairy product consumption on the other were observed. We studied the association between these products, calcium and colorectal cancer prospectively in the ongoing Netherlands Cohort Study (NLCS) on diet and cancer.[27]

Methods

The cohort characteristics and the method for cancer follow-up have been described.[27] Briefly, the study was initiated in 1986 when 58,279 men and 62,573 women aged 55 to 69 completed a self-administered baseline questionnaire inquiring after food habits and potential confounding factors. Following the case-cohort approach for analysis of the data, a subcohort of 3,500 persons (1,688 men, 1,812 women) was randomly sampled from the baseline cohort and followed up for vital status to estimate the person time contributed by the entire cohort. No subcohort members were lost to follow-up. Incident cancer cases occurring in the cohort were identified by record linkage to cancer registries and a nation-wide pathology register.[28] The analysis in this report was restricted to 3.3 years of follow-up from September 1986 (baseline) to December 1989. The completeness of cancer follow-up was estimated to be 95%.[29]

Food frequency questionnaire

The semi-quantitative food frequency questionnaire included 150 food items concentrating on habitual food and beverage intake during the preceding year.[30] The

daily amounts of 9 dairy items were estimated from the number of glasses (milk, chocolate milk, buttermilk), number of cups (yogurt, curds, custards with and without dietary fiber) or number of sandwiches filled (cheese and low-fat cheese). In addition, consumption of cheese as a meat substitute with the hot meal was asked for.

The questionnaire was validated against a 9-day dietary record.[30] The sex- and energy- adjusted Pearson coefficients of correlation for dietary calcium was 0.62. The Spearman coefficient of correlation for milk and milk products was 0.60 and for cheese 0.61. To minimize observer bias in coding and interpretation of the data, questionnaires were key-entered twice and processed blinded with respect to case/subcohort status.

Data analysis

Subjects who reported a history of cancer other than skin cancer in the baseline questionnaire were excluded. A total of 3,346 subcohort members (1,630 men, 1,716 women) and 478 incident cases (312 colon, 166 rectum; 258 men, 220 women) remained available for analysis. Furthermore, 7% of these subjects were excluded because of incomplete or inconsistent dietary data.[29] Therefore, 3,111 subcohort members and 443 colorectal cancer cases (293 colon, 150 rectum), 12 of which were members of the subcohort, were included in the analysis. As subclinical symptoms of colorectal cancer may influence dietary habits before diagnosis, data were analyzed without taking the first year of follow-up into consideration (3,083 subcohort members, 215 colon cancer and 111 rectal cancer cases).

Categories of dairy product intake were based on the subcohort distribution with non-users taken as the reference. Additional analyses were conducted excluding non-users and taking the lowest category of intake as the reference. For nutrient intakes, quintiles based on the subcohort distribution were used in the analysis. Since subjects tend to skip items they do not consume,[31] persons with missing data on a relevant dairy product were treated as non-users. We verified this assumption by conducting analyses including a variable indicating those with missing data on one of the specific foods.

In this cohort, the major fermented dairy products were buttermilk (39% of total consumption in grams), yogurt (39%), fat cheese (16%) and curds (6%). Because of their low contribution, curds are not taken into account in analyses on products. The fermented milks (yogurt and buttermilk) which differ from cheese with respect to their consumption pattern, were treated as one food group. With respect to cheese, analyses were restricted to full-fat cheese (95% hard cheese) which accounts for about 90% of cheese consumption in this cohort.

In this population, the consumption of yogurt and buttermilk was positively, although not strongly, correlated (r = 0.13).

Mean daily nutrient intake was calculated from mean food intakes using the computerized Dutch food composition table.[32] Energy-adjusted nutrient intakes were based on residuals from the regression of nutrient intake on total energy intake.[33] Since, exact amounts of calcium in the various supplements used were not available, calcium supplement use (alone (91%) or in combination with vitamin D) is examined as a dichotomous variable (use yes or no). Since less than 1% of the subcohort uses specific vitamin D supplements, these supplements were not taken into account in these analyses.

The case-cohort approach was used for data analysis.[34] Specific programs were developed to account for the additional variance introduced by estimating the person time-at-risk for the subcohort instead of for the entire cohort.[29]

Apart from the known risk factors for colorectal cancer (age, family history of large bowel cancer, history of gallbladder surgery[35]), the following variables were evaluated as potential confounders using multivariate models: smoking, level of education, any vitamin supplement use, intake of energy and energy adjusted intake of fat and dietary fiber. Cases with missing data on covariates were excluded from the multivariate analyses.
Vitamin D intake through sun exposure and from foods could not be adequately assessed and was not taken into account in these analyses.

In addition, analyses were conducted for colon and rectal cancer separately.

Results

Daily intake of yogurt and buttermilk together (median) ranged from 0 g/day in the lowest to 267 g/day in the highest category of intake. For hard cheese consumption, the intake ranged from 0 in the lowest to 37 g/day in the highest category. Intake of calcium ranged from 546 mg in the lowest quintile to 1,344 mg in the highest quintile; after energy adjustment these medians were 581 and 1,286 mg/day, respectively.

Table 8.1 shows various characteristics of subcohort members according to high and low intake of fermented milk and calcium.

Those who never consumed yogurt or buttermilk did not differ largely regarding age, energy intake and calcium/ phosphate ratio from those in the highest category of intake. Energy-adjusted intake of fat was higher, while energy-adjusted dietary fiber intake was lower in the lowest category of fermented milk consumption.

Table 8.1   Characteristics of subcohort members (n = 3,111) according to high and low intake of fermented milk[a] and calcium.

Variables	Fermented milk[a]		Calcium	
	Non-users	Highest category of intake	Lowest quintile of intake	Highest quintile of intake
Age ± SD	61.8 ± 4.2	61.5 ± 4.2	61.5 ± 4.1	61.2 ± 4.2
Gender (% men)	60.6	39.2	49.0	49.0
Dietary factors (mean ± SD)				
Fermented milk (g/day)	0	303 ± 116	34 ± 51	165 ± 163
Hard cheese (g/day)	17 ± 18	21 ± 19	8 ± 8	33 ± 24
Unfermented milk (g/day)	114 ± 162	91 ± 134	28 ± 49	233 ± 204
Calcium (mg/day)[b]	814 ± 281	1,146 ± 272	633 ± 135	1,300 ± 248
Energy (kJ/day)	8,102 ± 2,282	7,967 ± 2,100	6,738 ± 1,790	9,356 ± 2,311
Total fat (g/day)[b]	86 ± 17	79 ± 16	85 ± 15	82 ± 18
Dietary fiber (g/day)[b]	26 ± 7	29 ± 7	26 ± 6	28 ± 8
Calcium/phosphate ratio	0.6 ± 0.1	0.7 ± 0.1	0.5 ± 0.1	0.8 ± 0.1
Medical and life-style factors				
Body mass index (kg/m²)	25.0 ± 3.2	25.3 ± 3.2	25.1 ± 3.3	24.9 ± 2.9
Gallbladder surgery (%)	7.6	11.7	10.1	9.4
Family history of colorectal cancer (%)	5.0	5.7	5.1	5.5
High level of education (%)	11.4	15.4	10.0	13.3
Current smokers (%)	42.1	21.6	35.6	27.9
Calcium supplement users (%)[c]	1.8	5.5	2.2	4.8

[a]   Buttermilk and yogurt.
[b]   Adjusted for total energy intake by regression analysis.
[c]   Includes only calcium (91%) or calcium in combination with vitamin D or magnesium.

Women consumed yogurt and buttermilk more frequently than men (Table 8.1; median daily intake among users: women 96 g, men 79 g), whereas cheese consumption was similar for both genders (median daily intake among users 19 g). Body mass index and a positive family history for colorectal cancer did not differ appreciably between the two categories of consumption. Among the highest category of consumption, a history of gallbladder surgery was reported more frequently, and educational level was higher. The non-user group included more current smokers. Calcium supplements were used more frequently by those with the highest consumption.

Hard cheese consumption was positively associated with energy intake and educational level (data not shown).

Table 8.2   Relative rates of colorectal cancer according to consumption of dairy products.[a]

Average daily intake (g)	Subcohort members	Cases	Relative rates (95% CI)		χ² for trend[b] (p-value)
			Age-, gender-adjusted	Multivariate[c]	
Fermented milk[d]					
non-users	719	85	1.00	1.00	0.10 (0.75)
< 30	561	49	0.82 (0.56-1.20)	0.83 (0.56-1.21)	
30-90	569	67	1.07 (0.76-1.52)	1.10 (0.77-1.56)	
90-180	732	76	0.91 (0.65-1.28)	0.93 (0.66-1.31)	
≥ 180	502	49	0.90 (0.61-1.32)	0.89 (0.60-1.33)	
Hard cheese					
non-users	394	48	1.00	1.00	0.32 (0.57)
< 15	1,173	104	0.69 (0.48-0.99)	0.67 (0.47-0.97)	
15-30	860	107	0.90 (0.62-1.30)	0.94 (0.65-1.37)	
≥ 30	656	67	0.82 (0.55-1.21)	0.88 (0.59-1.33)	
Unfermented milk[e]					
non-users	1,126	130	1.00	1.00	0.70 (0.40)
< 120	819	77	0.79 (0.58-1.07)	0.81 (0.59-1.09)	
120-240	785	83	0.90 (0.66-1.21)	0.90 (0.67-1.22)	
> 240	353	36	0.85 (0.57-1.26)	0.86 (0.57-1.29)	

[a]   Excluding first year of follow-up.
[b]   Multivariate model.
[c]   Adjusted for age, gender, family history of colorectal cancer, intake of energy, energy-adjusted intake of fat and dietary fiber, Body mass index, history of gallbladder surgery.
[d]   Buttermilk and yogurt.
[e]   Whole milk and skim/low-fat milk.

Calcium intake was positively associated with energy intake, but no important associations of calcium were observed with age, gender, energy-adjusted intake of fat and dietary fiber, body mass index, family history of colorectal cancer, history of gallbladder surgery, educational level and smoking status (Table 8.1). The calcium/phosphate ratio was higher and calcium supplements (alone or in combination with vitamin D) were more frequently used among those with the highest dietary calcium intake. Previous publications provide differences between cases and subcohort members for several non-dairy variables.[36,37]

Table 8.2 shows the relative rates (RR) of colorectal cancer according to dairy product consumption. After adjustment for age and gender, consumption levels for fermented milk, cheese and unfermented milk were weakly inversely related to colorectal cancer risk. However, for fermented and unfermented milk, none of the category-specific relative rates nor trends were statistically significant. For yogurt and buttermilk separately similar non-significantly decreased risks were observed: RR for highest vs. lowest intake was 0.89 (95% CI = 0.65-1.21) for yogurt and 0.94

(95% CI = 0.68-1.30) for buttermilk. The associations with fermented dairy products tended to be most pronounced in women: RR for highest vs. lowest intake was 0.81 (95% CI = 0.46-1.44) for fermented milk and 0.61 (95% CI = 0.34-1.09) for hard cheese.

Adjustment for other potentially confounding variables in multivariate analysis did not change the results appreciably (Table 8.2). Education level, supplement use and smoking status were not included in the models since they did not affect the estimates. Additional adjustment for total calcium intake or inclusion of cases diagnosed during the first year of follow-up did not change the results either (data not shown).

The inverse association with fermented milk was restricted to colon cancer (multivariate RR for highest vs. lowest intake: colon = 0.70, 95% CI = 0.43-1.15, χ^2 trend = 0.23; rectum = 1.38, 95% CI = 0.74-2.57, χ^2 trend = 0.25).

Former analyses assume that those who skipped relevant dairy items in the questionnaire were non-users. When analyses were repeated, including an indicator variable for these subjects, RR estimates for fermented milk, cheese and unfermented milk did not change appreciably (RRs 0.90, 0.85 and 0.95 respectively). Excluding non-users from the analyses and choosing the lowest category of consumption as the reference altered the RRs slightly (RRs 0.96, 1.21 and 1.10 respectively).

Table 8.3 presents the observed relative rates for energy-adjusted dietary calcium intake and calcium from different dietary sources. After adjustment for age and sex and in multivariate models, total dietary calcium and calcium from fermented dairy products were not strongly associated with colorectal cancer risk, while calcium from unfermented dairy products tended to be inversely and calcium from non-dairy products positively associated. The non-significant inverse association with calcium from unfermented milk and the positive association with non-dairy calcium were most pronounced for rectal cancer (RR highest vs. lowest quintile for unfermented dairy calcium = 0.55, 95% CI = 0.30-1.04, χ^2 trend = 4.57; RR for non-dairy calcium = 2.32; 95% CI = 1.02-5.28, χ^2 trend = 5.67).

Calcium supplement use was not associated with risk (multivariate RR of users vs. non-users = 0.95, 95% CI = 0.50-1.78).

When analyses were conducted in terms of the calcium/phosphate ratio (RR per difference of 0.1 = 0.94, 95% CI = 0.85-1.04) or stratified according to gender, results remained similar (data not shown).

Table 8.3   Relative rates of colorectal cancer for quintiles of dietary calcium intake.[a]

Nutrient	Level of consumption					χ^2 for trend
	Q1	Q2	Q3	Q4	Q5	(p-value)
Total dietary calcium[b]						
number of cases	98	89	87	81	88	
number in subcohort	623	619	622	627	620	
median intake in subcohort, mg/day	596	768	893	1,032	1,288	
age- and gender-adjusted RR	1.00	0.83	0.96	0.92	0.93	0.03 (0.87)
multivariate RR[c]	1.00	0.84	0.96	0.93	0.92	0.02 (0.89)
(95% CI)		(0.58-1.22)	(0.67-1.39)	(0.64-1.36)	(0.64-1.34)	
Calcium from non-dairy products						
number of cases	91	82	95	93	82	
number in subcohort	621	625	622	621	622	
median intake in subcohort, mg/day	238	284	318	356	417	
age- and gender-adjusted RR	1.00	1.14	1.51	1.28	1.25	1.77 (0.18)
multivariate RR[c]	1.00	1.27	1.82	1.64	1.77	6.56 (0.01)
(95% CI)		(0.83-1.92)	(1.21-2,74)	(1.05-2.56)	(1.08-2.90)	
Calcium from fermented dairy products[d]						
number of cases	81	87	94	95	86	
number in subcohort	625	620	621	628	617	
median intake in subcohort, mg/day	64	181	287	394	634	
age- and gender-adjusted RR	1.00	0.96	1.20	1.10	1.07	0.41 (0.52)
multivariate RR[c]	1.00	1.01	1.29	1.18	1.14	0.98 (0.32)
(95% CI)		(0.69-1.48)	(0.89-1.88)	(0.80-1.72)	(0.77-1.68)	
Calcium from unfermented dairy products						
number of cases	110	85	78	93	77	
number in subcohort	622	625	616	624	624	
median intake in subcohort, mg/day	45	150	238	345	540	
age- and gender-adjusted RR	1.00	0.78	0.70	0.81	0.72	2.65 (0.10)
multivariate RR[c]	1.00	0.78	0.69	0.79	0.71	2.60 (0.11)
(95% CI)		(0.54-1.11)	(0.48-1.01)	(0.55-1.14)	(0.48-1.05)	

[a]   Excluding first year of follow-up.
[b]   Adjusted for total energy intake by regression analysis. Total dietary calcium does not include calcium supplements.
[c]   Adjusted for age, gender, family history of colorectal cancer, intake of energy, energy-adjusted intake of fat and dietary fiber, body mass index, history of gallbladder surgery.
[d]   Yogurt, buttermilk, cheese, curds.

Discussion

These data do not support a protective role of fermented dairy products or calcium in colorectal cancer risk. Although risks of colon and rectal cancer were inversely associated with fermented milk consumption and calcium intake from unfermented dairy products respectively, none of the relative rates were significantly different from unity.

A potential drawback of this study is the short follow-up period, i.e. 3.3 years. Especially when gastro-intestinal complaints in the time span before diagnosis would have changed dairy product intake, this could have altered the associations. However, the similarity of results observed in the data with and without cases diagnosed within the first year of follow-up does not support this possibility. Our questionnaire inquired after dietary patterns in the preceding year and might not be representative over a longer period of time. However, this is a population of elderly people, who are usually considered to have stable food patterns. This stability is confirmed by a repeatability study, in which the food frequency questionnaire was readministered in five consecutive years after baseline assessment. It indicated fairly stable nutrient intakes over time (unpublished data). Furthermore, the consumption of these sour fermented milk products is strongly related to preference, which makes it reasonable to assume that the intake of these products does not change dramatically over the years.

The absence of an observed association is not likely to be explained by insufficient contrast in the population. In our cohort, the intake of yogurt and buttermilk varied from zero in the lowest category to a median of 267 g/day in the highest one according to the questionnaire data; and for cheese from zero in the lowest to 37 g/day in the highest category. Despite a lower per capita intake[24] and presumably a smaller range of intake in the United States, in two American colon cancer case-control studies[16,20] a significant protective effect was found for cultured milk and yogurt. However, no important associations between fermented dairy products and colorectal polyps were found in the Nurses' Health Study and the Health Professionals Follow-up Study, two American prospective studies.[23] In a Dutch breast cancer case-control study (133 cases, 289 population controls), women with a daily intake of 225 g of fermented milk had a 45% lower risk (OR = 0.55, 95% CI = 0.24-1.27) than non-users.[25] An odds ratio of 0.49 (95% CI = 0.14-1.76) was found for those women who consumed 20 to 40 g of cheese per day compared to non-users.[25] In a Dutch case-control study on pancreatic cancer, an inverse association with fermented milk products (highest vs. lowest quintile of intake OR = 0.37, $p < 0.05$) and cheese (OR = 0.44, $p > 0.1$) was observed.[26]

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Contents

Integration of results and epidemiological considerations

Integration of results

Epidemiological considerations
End-points
Assessment of exposure
Variation of exposure and biological relevance
Effect modification
Power consideration

Integration with the literature

Fermented dairy products
Analytical epidemiological studies
Experimental studies

Calcium
Analytical epidemiological studies
Human Experimental studies

Concluding remarks

Integration of results and epidemiological considerations

Integration of results

In this thesis the results of four epidemiological studies are described in which the associations between fermented dairy products, calcium and colorectal adenomas or colorectal cancer were examined. The main results and characteristics of these studies are summarized in Figure 9.1 and Table 9.1 respectively.
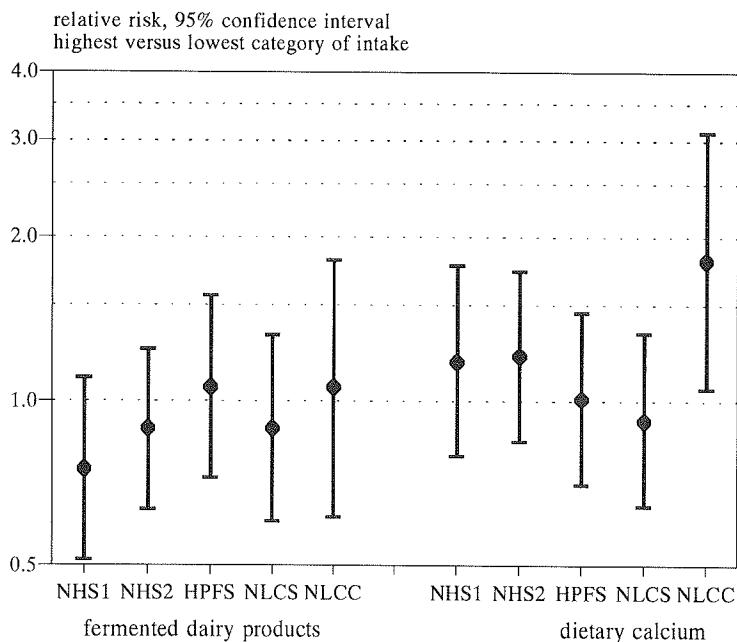
Figure 9.1   Fermented dairy products, calcium and colorectal cancer risk in the four epidemiological studies described in this thesis (NHS1 = Nurses' Health Study 1984-1988; NHS2 = Nurses' Health Study 1980-1988 and Health Professionals Follow-up Study; NLCS = Netherlands Cohort Study; NLCC = Netherlands Case-Control study).

For fermented dairy products non-significant inverse associations were observed in the two follow-up periods of the Nurses' Health Study (Chapter 3) for colorectal adenomas and in the Netherlands Cohort Study (Chapter 8) for colorectal cancer, while in the Health Professionals Follow-up Study (Chapter 3) and the Netherlands Case-Control Study (NLCC) non-significant positive associations were observed (Figure 9.1). Dietary calcium was non-significantly inversely related to risk in the Netherlands Cohort Study, while no association was found in the Health Professionals Follow-up Study, a non-significant positive association was observed

in both follow-up periods of the Nurses' Health Study and a significant increased risk with increasing calcium intake was found in the Netherlands Case-Control Study.

The overall inverse variance weighted estimate of relative risk in these four studies, excluding Nurses' Health Study 2, was 0.91 (95% CI = 0.74-1.12) for the highest vs. the lowest category of fermented dairy product consumption and 1.10 (95% CI = 0.90-1.35) for the highest vs. lowest category of dietary calcium intake. Overall, the suggested protective effect of fermented dairy products and dietary calcium on colorectal adenomas or cancer risk is not supported by the results of these studies.

These null findings might reflect the underlying association, but might also result from a failure to observe a truly existing inverse association. Such a failure might be attributed to the end-point under study, the methodology of exposure assessment, the variation of exposure in the study population and the biological relevance, confounding or effect modification or a lack of statistical power.[1] The purpose of this chapter is to discuss these possible explanations for our null findings (see 'Epidemiological considerations'). In addition, the external validity of these studies is evaluated by comparing our findings with the results of other analytical epidemiologic studies and human experimental research (see 'Integration with the literature').

The studies described in this thesis represent three different end-points (distal adenomas, colon and rectal cancer), three different epidemiological study designs (cohort, case-subcohort, case-control), two different methods of exposure assessment (semi-quantitative food frequency and dietary history) and, since these studies were conducted in different populations (US and the Netherlands), different ranges of fermented dairy product intake (Table 9.1). The studies can be compared to a reasonable degree with respect to definition of exposure and adjustment for potential confounding variables. The advantages and disadvantages of these studies are described in more detail in the corresponding chapters (Chapter 3 to 8).

Epidemiological considerations

End-points
As discussed in Chapter 2, colorectal carcinogenesis is a process of several stages in which adenomatous polyp formation precedes the formation of the malignant tumor. The mechanisms by which fermented dairy products and calcium might reduce colorectal cancer risk could involve earlier stages as well as later stages in the polyp-cancer sequence and may differentially affect the various sites of the colorectal tract (Chapter 2).

Adenomatous polyps can serve as an intermediate end-point for the study of determinants of colorectal cancer, which shortens the period between dietary assessment and the onset of the disease. If diet affects polyp formation, stronger associations with fermented dairy products and calcium might be found when studying these precursor lesions.

Table 9.1   Characteristics of the four studies described in this thesis.

	Nurses' Health Study 1	Nurses' Health Study 2	Health Professionals Follow-up Study	Netherlands Cohort Study	Netherlands Case-Control Study
Type of study	cohort	cohort	cohort	cohort	case-control
Population	US, endoscopied nurses (women)	US, endoscopied nurses (women)	US, endoscopied health professionals (men)	Netherlands, general population	Central and East Netherlands, general population
End-point	distal colon, rectum, adenoma	distal colon, rectum, adenoma	distal colon, rectum, adenoma	colon, rectum, carcinoma	colon carcinoma
Years of follow-up	4	8	4	3.3	-
Number of cases/ controls or cohort	283/6,988	350/8,575	331/9,159	326/3,083	232/259
Dietary method[1]	sqffq i = 121	sqffq i = 61	sqffq i = 131	sqffq i = 150	dietary history i = 289
Median (per day) highest quintile or quartile of intake[2]	ferm.dairy[3]: 129 g calcium: 1,550 mg	ferm.dairy[3]: 134 g calcium: 1,160 mg	ferm.dairy[3]: 111 g calcium: 1,500 mg	ferm.dairy[4]: 267 g calcium: 1,288 mg	ferm.dairy[5]: 335 g calcium: 1,712 mg
Adjusted for	age, energy, bmi,alcohol, folate, sat.fat, fiber, fam.history endoscopies	see NHS1	see NHS1	age, gender, fat energy, fiber, bmi, fam.history cholecystectomy	age, gender, energy, fat, fiber, fam.history, cholecystectomy, alcohol, vit.C

[1]   sqffq, semi-quantitative food frequency questionnaire.
[2]   among controls or (sub)cohort.
[3]   yogurt, sour cream, cream cheese, cottage cheese, hard cheese.
[4]   yogurt, buttermilk.
[5]   yogurt, buttermilk, sour cream, curds, kefir, other fermented milk drinks.

Since we assessed the association between these exposures and the risk of both adenomas as well as cancer, it is unlikely that an important effect was missed just because of an irrelevant time window in colorectal cancer aetiology. Despite these biological and methodological considerations, no differences in associations with fermented dairy products and calcium were observed for colorectal adenomas and colorectal cancer: for both end-points no important associations were found.

Based on experimental and epidemiological studies suggesting partially different aetiologies for cancer of the colon and rectum, we examined the association with risk for these different subsites separately. Slight differences were observed between colon and rectum cancer in the Netherlands Cohort Study: fermented dairy product consumption was more strongly associated with colon cancer risk, while the intake of calcium from unfermented dairy products was inversely associated with rectal cancer.

Cancer of the proximal and distal sites of the colon might be explained by different aetiological factors as well. However, since sigmoidoscopy or endoscopy usually do not reach the proximal site of the colon, this site was not taken into account in studying adenoma risk. Because of power considerations, we did also not explore differences between subsites in the studies on colon cancer.

Thus, the null findings of these studies are not likely to be explained by the choice of the end-point under study. However, the potential differences between colon and rectal cancer, and among different subsites within the colon, need further study. A longer duration of follow-up of the Netherlands Cohort Study will provide opportunities to explore these differences in more detail and to study different subsites within the colon. These additional analyses will be conducted after 5 years of follow-up supported by a grant from the Netherlands Digestive Diseases Foundation.

Assessment of exposure

The main hypothesis in this thesis was studied in three prospective studies and one retrospective study. Prospective studies are less prone to potential sources of methodological bias in exposure assessment, such as recall and selection bias, associated with retrospective case-control investigations. Because in prospective studies exposure information is collected before diagnosis, illness is not very likely to affect recall of previous habits. In Chapter 5 it is discussed that recall bias might be especially important in a case-control study when cases, as a result of their disease, report their usual intake before complaints started or diagnosis occurred differently from the control group. This problem is becoming increasingly relevant because of the widespread publicity about diet and cancer. However, since dairy products have a good image in the Netherlands it is thought to be unlikely the

cases, whether or not intentionally, have overestimated their calcium intake to 'explain' their illness. Furthermore, dietary patterns might have been adapted to gastro-intestinal distress or food aversions associated with therapy. Since current dietary patterns influence the recall of past diet,[1] such changes might bias the results importantly. In our case-control study, none of those who replied to suffer from gastro-intestinal distress reported to have changed their consumption of dairy products (Chapter 2).

The second cause of a lack of internal consistency in a case-control study could be biased sampling of cases and controls, especially when participation rates are low. In our case-control study 60% of the invited cases 57% of the controls were willing to be interviewed. In Chapter 4 it is argued that the possible selection of cases and controls due to non-response will strengthen an inverse association. However, those who completed the non-response questionnaire may not be representative of the total of non-responders (Chapter 4). The fact that more cases with a relatively good prognosis have been included in the case-control study is not likely to have influenced the results considerably, since consumption of fermented dairy products and intake of calcium were not related to Dukes' status (Chapters 4 and 5). Furthermore, recruitment of controls by the general practitioner of the cases made the control group more comparable to the general population with respect to socio-economic level than a control group recruited via municipal registries. This might explain differences with previous Dutch case-control studies on breast and pancreas cancers respectively[2,3] (Chapters 4 and 5).

A methodological bias which might be important both in retrospective and in prospective designs, is that the method of dietary assessment is not sufficiently precise to measure differences that truly exist.[1] Evaluations of the validity and reproducibility of the semi-quantitative food frequency questionnaires used in the American and the Dutch prospective studies indicated that these questionnaires provided useful information about the consumption of dairy products and calcium (Chapters 3 and 8). The dietary history questionnaire in the case-control study was an extended version of a previously used validated questionnaire (Chapter 4). The median intake of calcium among controls in the case-control study was higher than in the Netherlands Cohort Study and higher than the estimated mean intake in the general Dutch population as assessed by a two-day dietary record.[4] This might be a consequence of the extensive number of questions addressing dairy products. In the American studies the questionnaires used in the four-year follow-up included 11 dairy items, in the Netherlands Cohort Study 9 dairy items were asked for, while in the case-control study the consumption of all common dairy items used during different meals was assessed with a maximum of about 25 products. Furthermore,

questionnaire. Sizes of major cups and utensils were measured. The questionnaire used in the case-control study was interview-based and not self-administered. This is believed to contribute to a higher degree of internal consistency in the reported food pattern, resulting in more precise estimates of consumption.[5]

With respect to the time window of dietary assessment, it is clear that the American studies covered a period preceding tumor formation. Nevertheless, when the onset of the disease occurred during childhood, we may have missed a truly existing association. It seems however unrealistic to assume that valid information could have been gathered since studies conducted to date suggest that diet may be recalled with acceptable levels of misclassification up to approximately 10 years. Moreover, the fact that colorectal cancer rates rise consistently with migration and westernization[6] suggest that dietary factors might be especially important in adult life.

Thus, although some degree of recall bias and selection bias can never be completely ruled out in a retrospective study, the errors associated with assessment of fermented dairy product consumption and calcium intake in these studies are not likely to be influenced by methodological biases.

Variation of exposure and biological relevance
To observe a possible statistically significant inverse association between fermented dairy products, calcium and colorectal cancer, sufficient variation in consumption levels for fermented dairy products and calcium in the study population is required. Moreover, it must be considered whether the absolute level of intake is of biological relevance and whether negative confounding exists.

The consumption of fermented milk, such as yogurt and buttermilk, is strongly related to taste: the fact that the sour taste is liked or disliked contributes to variation in consumption level. Since fermented dairy products are integrated in the traditional Dutch food pattern and the per capita consumption of these products is much higheer than in the US (Chapter 2), it might be worth-while to study this subject in the Netherlands. Although consumption of fermented dairy products indeed differed between the Dutch and the American studies (Table 9.1), the intake of calcium did not differ appreciably between populations in the prospective studies (Table 9.1). However, despite the higher and more heterogeneous consumption of fermented dairy products in the Netherlands, the estimates of the four studies did not differ appreciably.

It is questionable whether the consumption of fermented dairy products or the intake of calcium was high enough to exert a biological effect. Our ecologic study (Chapter 2, Figure 2.2) suggested that the range of consumption of fermented milk

able to observe a relative risk less than 0.5. Moreover, the level of consumption of fermented dairy products in the Dutch studies was comparable to that in a previous study on breast cancer in which a protective effect was observed. However, based on the results of experimental studies, it may be inferred that still a higher and more frequent consumption is necessary to influence the colonic flora (see 'Experimental studies'). With respect to dietary calcium the range in intake in our studies should have been high enough to show a decrease in risk as calculated by Garland's[7] and Newmark's[8] groups. They estimated an intake of 1,200-1,500 mg calcium per day to be sufficient to neutralize bile acids and free fatty acids in the colonic lumen with an intake of about 150 g fat and 1,500 mg phosphate per day. The mean intake of fat in our studies was about 80 to 90 g per day, whereas the mean intakes of phosphate and calcium ranged from 1,200 to 1,700 mg in the highest categories. This implies that a substantial proportion of participants in our studies should have been consuming an adequate amount of calcium to experience the biological effects. On the other hand, if calcium influences cell proliferation directly, which is the second hypothesized mechanism as described in Chapter 2, the intracellular concentration might be more important than the intraluminal calcium content. In this respect, vitamin D status might be of major importance. However, in the American cohorts, vitamin D intake did not influence the results on colorectal polyps (Chapter 3; and see 'Effect modification'). In the Dutch studies vitamin D exposure was not assessed (Chapters 5 and 8).

The biological relevance might also depend on the specific strains of bacteria used for the fermentation process. The bacterial strains used to culture milk might differ among countries, states and products. In the Netherlands, *Lactobacillus bulgaricus, Streptococcus thermophilus, S. lactis* and *S. tremor* are mainly used to produce yogurt and buttermilk. These strains are used in some parts of the US as well, but in other parts *Lactobacillus acidophilus* is more frequently used for fermentation. Since several strains might not survive the gastro-intestinal tract and possibly only viable bacteria exert an effect on the bowel flora (see 'Experimental studies'), the differences in findings among epidemiological studies might be explained by differences in fermented dairy products studied. However, in a Dutch case-control study on breast cancer, an inverse association was observed for yogurt, buttermilk and cheese, whereas cheese mainly contains unviable bacteria. In the four studies no differences among the different fermented dairy products were found.

Considering the population-specific confounding variables, it was expected that, since the consumption of fermented milk is rare sparse in the US, the consumption of these products might be related to other health conscious habits. However, in the two Dutch studies the use of fermented dairy products was also clearly related to

health consciousness (e.g. less smoking, less alcohol use, more supplement use, a higher consumption of vegetables). Adjustment for these potential confounding variables did not influence the estimates importantly, however.

In conclusion, variation in intake, absolute consumption levels for fermented dairy products and calcium or confounding are not likely to explain the null findings of our studies. However, these results do not exclude the possibility that inverse associations might still be found with higher doses of dairy products, or with products cultured with other bacterial strains.

Effect modification

Interaction with other dietary variables might have influenced the results. As described in Chapter 2, especially a high dietary phosphate/calcium ratio or the vitamin D status might modify the associations between calcium and colorectal cancer risk. Furthermore, if calcium exerts an effect on colorectal carcinogenesis by binding bile acids and free fatty acids, whose concentrations in the colonic lumen raise with higher saturated fat intake, the observed association may depend on the fat content of the diet. Finally, dietary fiber might modify the effect by binding calcium. The possible effect modifications were studied in the American studies by assessing the association with calcium at different levels of phosphate, vitamin D, saturated fat and dietary fiber intake. It appeared that the associations between calcium and colorectal polyp risk did not differ at different levels of intake of these dietary variables. Moreover, a high phosphorus intake relative to the intake of calcium was evaluated in all four studies. The ratio was not associated with adenoma or carcinoma risk.

Apart from mechanistic interactions, sex and/or age may be potential effect modifiers. Incidence rates differ between men and women over categories of age (Chapters 2 and 6). The four studies differ in gender and age distribution: at baseline the Nurses' Health Study included women only, who were mainly premenopausal, while the Health Professionals Follow-up Study included only men, aged between 40 and 75. The two Dutch studies both included men and women: the Netherlands Cohort Study included middle-aged men and women, while in the Netherlands Case-Control Study men and women younger than 76 years at diagnosis were included (mean age about 61). Differences were observed between genders: consumption of yogurt and buttermilk was significantly higher among women. Moreover, the association between consumption of fermented dairy products and colorectal adenoma or cancer risk differed between genders: among women (non-significant) inverse associations were found, while for men these associations tended to be positive. For dietary calcium differences between genders were not consistently found (Figure 9.2).

In the Netherlands Case-Control Study a significant difference between men and women was observed for fermented dairy products. Additionally, gender interactions were observed for fruit and meat consumption (Chapter 7) and different associations with parity and age at first child birth were found for men and women (Chapter 6).
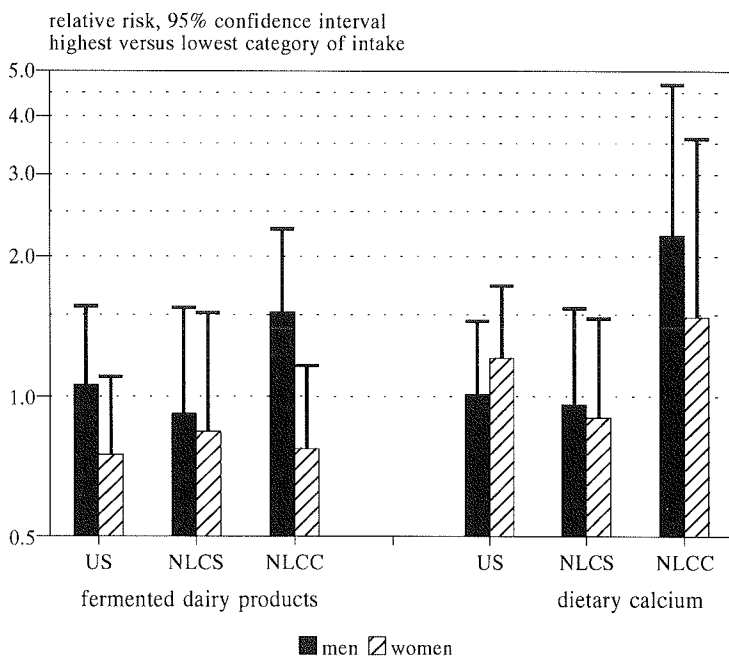
Figure 9.2   Gender-specific associations between colorectal cancer and fermented dairy products and dietary calcium (US = Nurses' Health Study 1 and Health Professionals Follow-up Study; NLCS = Netherlands Cohort Study; NLCC = Netherlands Case-Control study).

Differences in the strength of associations between men and women might be attributed to differences in internal validity of the studies for both sexes or by actual biological differences. Considering the internal validity of the studies, men might report their diet less accurately than women, which might attenuate the relative risks. However, in a review of dietary recall methods by Friedenreich et al., it was concluded that men and women recall their previous diet with a similar accuracy.[5] Furthermore, in the Netherlands Case-Control study this difference in reporting should be minimal since women, who normally cook the main meal in this older age group, were present during their husbands' interview. Moreover, the Dutch National Food Consumption Survey data support the finding that the consumption of fermented dairy products is higher among women.[9]

Finally, biological explanations might be proposed as well. As described in

Our findings for cheese consumption are not in line with one of the two prospective studies and three of the six case-control studies addressing hard cheese consumption in which positive associations for colorectal cancer were found (Chapter 2). Significant positive associations were observed for rectal cancer only. In most of the studies addressing cheese consumption, the analyses were not adjusted for fat intake. Our null findings for cheese are especially interesting since cheese is a high-fat food item and an increased risk with dietary fat was observed in the Nurses' Health Study, the Health Professionals Follow-up Study and the Netherlands Case-Control Study.

The prospective study by Phillips et al.[13] found a significant positive association with cheese consumption for men, while a non-significant inverse association was found for women. Although this might support a hormonal effect of lactobacilli, it might also point to other habits related to cheese intake. Since none of the other studies on fermented dairy products report results of analyses stratified by gender firm conclusion cannot be drawn.

The results of the Netherlands Cohort Study and the Netherlands Case-Control Study are not in line with the results of previous case-control studies on breast cancer[2] and pancreatic cancer conducted in the Netherlands.[3] Both studies observed an inverse association with consumption of fermented dairy products. Although the aetiology of colon cancer differs from that of breast cancer and pancreatic cancer, the inverse associations reported from these studies might be explained by the choice of the control group. Consumption of fermented dairy products is significantly higher in those with a higher socio-economic status. If the population-based control groups used in those studies included a higher proportion of more health-conscious, more educated and well-to-do persons, these studies could erroneously observe an inverse association with fermented dairy products. As described in Chapter 4, the socio-economic status distribution of the control group used in our case-control study was comparable to that of the general Dutch population. The additional control group we recruited via municipal registries, like in the other case-control studies, indeed included more persons with a higher socio-economic status.

Overall, the epidemiological evidence on fermented dairy products is too sparse to allow firm conclusions to be drawn. The available evidence, however, does not provide strong evidence for a clear preventive potential.

Experimental studies

Like epidemiological studies, experimental data on fermented dairy products or lactic acid bacteria are sparse and preliminary. As reviewed in Chapter 2, experimental studies on lactic acid bacteria focus especially on (1) modulations of

the colonic flora, (2) enhancement of the immune response and (3) mutagen absorption.

Several investigators explored the first hypothesis and endorse a decline in activity of several faecal enzymes. However, the role of these enzymes in the aetiology of colorectal cancer has not been substantiated, the mechanisms by which lactic cultures decrease enzyme activity is not known and a reliable measurement of faecal enzymes remains difficult.[14] It is not clear whether only strains that are resistant to gastric acidic conditions and remain viable in the colon exert an effect.[15,16] Most experimental studies used specific strains that are not normally present in commercially available fermented dairy products, which might explain why their results differ from our epidemiological studies. Furthermore, it is still questionable whether a daily dairy dose of lactic acid bacteria is large enough to influence colonic metabolism, since the number of bacteria in the colon is much larger. There is no evidence that lactobacilli can establish themselves in the intestinal tract and it could be that fermented milk needs to be ingested continuously, or at least regularly, to maintain a sufficient number of lactobacilli in the intestine.

Research on the other two hypothesized modes of action of lactic acid cultures is still very preliminary. It is not clear whether ingestion of large numbers of lactobacilli stimulates the human immune system more than the normal constant stimulus by the gut flora already does. Mutagen absorption studies are mainly in vitro studies whose physiological significance may be limited. There is no evidence for any specificity of lactobacilli in mutagen binding.

Overall, experimental data on commercially available fermented dairy products are limited and too preliminary to provide evidence for a protective effect of fermented dairy products on colorectal cancer risk.

Calcium

Analytical epidemiological studies
Two small retrospective studies have addressed the intake of dietary calcium and the occurrence of colorectal polyps (Chapter 2): odds ratios of 0.9[17] and 1.2[18] were reported. These results are in line with the findings in the Health Professionals Follow-up Study and the Nurses' Health Study respectively (Chapter 3).

Results on dietary calcium intake and colorectal cancer in the Netherlands Cohort Study are comparable with the results of 4 of the 6 prospective studies on colorectal cancer addressing dietary calcium intake (Chapter 2). Five of these studies were conducted in the US. Garland et al.,[19] whose study is the most cited study on

The assumption that a reduction of proliferation rate is related to a reduced risk of colorectal cancer has not been established; a reduction of proliferation rate may not be sufficient to reverse the carcinogenic process.[27]

Most published studies have investigated overall cell proliferation. A reduced proliferation rate is not supported by the largest placebo-controlled trial on calcium conducted to date, which included 193 cases with sporadic adenomas, and which has been completed very recently. However, the results do show an effect of calcium on the normalizing downwards shift of the proliferative zone (J.D. Potter, personal communication, 1994) as has also been reported previously by Lipkin et al. in an uncontrolled trial.[28]

The results of of the large trial mentioned above suggest that the mechanism by which calcium reduces colorectal cancer risk might be far more complex than previously proposed by Newmark et al.[8] who hypothesized that calcium binds toxic fatty acids and bile acids. For instance, in a recent study by Alder et al., faecal pH and deoxycholic acid concentrations tended to increase during calcium supplementation.[29]

However, even if the precise mechanism is still unknown and if cell proliferation is a biomarker for colorectal cancer risk, the current data indicate that a total of at least 2000 mg calcium per day would be necessary to decrease the proliferation of the mucosa importantly. If so, this dose largely exceeds the usual dietary dose. Therefore, the relatively low level of calcium intake in our epidemiological studies can still be compatible with our null findings or with the overall slightly inverse association reported in the literature.

Concluding remarks

The studies described in this thesis do not substantiate an important preventive potential for fermented dairy products and dietary calcium. However, it is still questionable whether our findings contradict the results of experimental studies. Human experimental studies are usually conducted with fermented dairy products which are commercially not available, while the dose of lactobacilli or calcium salts is much higher than the average intake in the population at large. Placebo-controlled studies with commercially available products and lower doses might help to explain the differences in findings between epidemiological and human experimental studies.

Experimental studies exploring different end-points should be conducted. Instead of overall proliferation rate, more emphasis should be placed on the potential effect of calcium to shift the zone of proliferation from one that includes the entire crypt

to one that is confined to the lower zone of the crypt. More evidence need to be gathered on whether hyperproliferation is indeed a suitable intermediate stage in colorectal carcinogenesis. Moreover, hyperproliferation is mainly assessed in rectal epithelium, while in the only experimental study focusing on the sigmoid part of the colon, a positive association with calcium was observed. Experimental as well as epidemiological studies should further explore differences in aetiology between colon and rectal cancer and between the different sites within the colon.

Large intervention studies are currently being undertaken in the US[30] and Europe[31] studying the effect of different dietary factors on either the occurrence or the recurrence of polyps. These studies might provide insight into the effect of diet on early events relevant to colorectal carcinogenesis. Fermented dairy products and dietary calcium might be studied in similar designs with an emphasis on different subsites within the large bowel. However, supplemention with large amounts of calcium might not be completely harmless. For instance, Paraskeva argued that caution is necessary with regard to dietary intervention studies.[32] He indicated that differential sensitivity of normal and malignant tissue to calcium *in vitro*[33] raises the possibility that levels of calcium inhibiting the growth of normal epithelium might provide a selective growth advantage of both adenoma and carcinoma cells over normal cells, and thereby promote carcinogenesis in the long run.[32] Although this hypothesis is not supported by human experimental studies, it might be important to explore this further before large-scale intervention takes place.

Differences between genders in colon cancer risk at specific subsites and the different associations observed for several dietary factors still provide interesting leads for research. Sex differences in colon physiology related to the bacterial flora should be explored more explicitly.

Several lines of inquiry hint at links between epidemiological risk factors and processes on the molecular level. Some of these are described in Chapter 2 of this thesis. Genetic susceptibility to colorectal cancer might be more important than one would expect. Although hypotheses lack specificity, the metabolism of certain carcinogenic compounds, such as the acetylation of arylamines from cooked meat, might be under genetic control.[34] If calcium indeed stimulates DNA repair,[35] this might be especially important for those who are genetically susceptible to DNA-damaging compounds. The fact that epidemiological studies have yielded weak associations and seemingly contradictory results might be explained by the simultaneous inclusion of colon cancers with distinct aetiologies as one single disease. Distinction of different types of colon cancer - those with and without ras mutations or those with a slow or a fast acetylator status - will help disentangle different aetiologies and might help explain the apparently inconsistent results of epidemiological studies, e.g. those addressing dietary calcium. Findings of

epidemiological studies based on molecular tools might strengthen the scientific rationale for dietary advice relevant to colorectal cancer prevention.

In conclusion, our findings in four epidemiological studies show a neutral effect of dairy products, in particular fermented dairy products, and calcium in colorectal cancer aetiology. Furthermore, the Netherlands Case-Control Study supports a protective effect of vegetables, especially in women consuming a diet rich in red meat. These results are in line with the dietary recommendations put forward by the Dutch Nutrition Council and the Dutch Cancer Society.

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Summary

Summary

Colorectal cancer is one of the most prevalent cancers in westernized countries; in the Netherlands, about 7000 new cases are diagnosed each year. Since mortality rates are still relatively high and no major improvements in treatment strategies are expected in the near future, prevention of colorectal cancer is of major importance.

Chapter 2 provides an overview of the current concepts in the aetiology of colorectal cancer. In the past decades several stages in the multistep process of colorectal carcinogenesis have been identified, and it appeared that colorectal cancer arises as a result of the mutational activation of oncogenes coupled with the mutational inactivation of tumor suppressor genes. The vast majority of colorectal tumors are thought to arise in adenomatous polyps. The risk of developing an adenoma or carcinoma might depend on both genetic and environmental factors. Diet is considered to be one of the most plausible environmental factors, since the large bowel is in direct contact with the digested foods and their metabolites. A widely accepted hypothesis is that a westernized high-fat, low-fiber diet increases the risk for colorectal cancer. In terms of foods, an increased risk has mainly been attributed to the consumption of red meat and a decreased risk to the consumption of vegetables and fruits.

Besides a higher consumption of vegetables and fruits, a higher consumption of fermented dairy products, such as yogurt, buttermilk and cheese, might provide a relatively simple and feasible dietary modification for colorectal cancer prevention. Experimental studies suggest that a protective effect of fermented dairy products might be attributable to either the presence of lactic acid bacteria or their calcium content. Certain strains of lactobacilli might positively influence the colonic flora, stimulate the immune response or bind mutagens. Calcium might reduce the proliferative activity of the colon mucosa indirectly by diminishing the proliferative stimulus of secondary bile acids and free fatty acids in the colonic lumen or directly by inducing cell differentiation. As discussed in Chapter 2, few epidemiological studies conducted to date have addressed the consumption of fermented dairy products, while the epidemiological studies on calcium are not conclusive.

This thesis describes the results of four epidemiological studies investigating whether the consumption of fermented dairy products and the intake of calcium could provide feasible tools to prevent colorectal cancer. The studies represent different end-points and levels of exposure, different study designs and dietary methodologies and different populations (Chapters 3 through 8).

Chapter 3 describes results of two of these four studies, The Nurses' Health Study (NHS; 98,464 women; 30-55 years at baseline), and The Health Professionals Follow-up Study (HPFS; 51,529 men; 40-75 years at baseline); both ongoing studies have been conducted at the Harvard School of Public Health in Boston, US. In these large prospective studies, the association between fermented dairy products, calcium and the occurrence of colorectal adenomatous polyps was investigated using a self-administered semi-quantitative food frequency questionnaire. Cases (331 men and 350 women) were patients with adenomatous polyps of the left colon or rectum diagnosed during four years (HPFS and NHS) or eight (NHS) years of follow-up; controls (9,159 men and 8,585 women) were persons with negative endoscopies. After adjustment for age, total energy, family history and saturated fat, fermented dairy product consumption was not related to the risk of adenomas (NHS: relative risk (RR) for highest vs. lowest cetgory of consumption = 0.8, 95% confidence interval (CI) = 0.5-1.1; HPFS: RR = 1.1, 95% CI = 0.7-1.6). Adjustment for other potential confounding variables did not change the results importantly. Calcium intake from dietary sources and supplements was unrelated to adenoma risk in men (multivariate RR for highest vs. lowest quintile of intake = 1.1, 95% CI = 0.8-1.7) and women (RR = 1.2, 95% CI = 0.8-1.7). No important differences were observed for dietary or supplemental calcium. Although no association with dietary vitamin D was found, vitamin D from supplements was slightly, but not significantly, inversely associated with risk among women only (RR = 0.7, 95% CI = 0.4-1.1).

The hypothesis was further explored in two Dutch studies since in the Netherlands the consumption of fermented dairy products is integrated in the traditional food pattern and the variation in consumption is sufficiently large. A population-based case-control study was conducted in the eastern and central parts of the Netherlands (Chapters 4 through 7). Dietary patterns were assessed among 232 colon cancer cases and 259 controls by means of a structured dietary history questionnaire. As described in Chapter 4, cases were recruited by surgeons in 14 cooperating hospitals. The cancer registries of the central and eastern parts of the Netherlands were used as a check of completeness and provided additional clinical and pathological information. Controls, frequency-matched to the cases by age (5-year intervals), gender, region and urbanization grade, were recruited randomly by the general practitioners of the participating cases. After adjustment for potential confounding variables, the consumption of fermented milk (odds ratio (OR) for highest vs. lowest category of consumption = 1.1, 95% CI = 0.6-1.8) and hard cheese (OR = 1.2, 95% CI = 0.7-2.1) were not significantly associated with risk of colon cancer (Chapter 5). Dietary calcium was positively associated with colon

cancer risk (multivariate OR = 1.8; 95% CI = 1.1-3.1). This unexpected finding is discussed in Chapters 5 and 9. The observed associations differed between men and women: positive associations were observed in men, while in women non-significant associations were found. Sex differences in colon cancer risk were also observed for the consumption of fruits and red meat, for parity and age at the birth of the first child (Chapters 6 and 7).

In line with previous studies, the consumption of vegetables was significantly associated with reduced colon cancer risk (OR = 0.4, 95% CI = 0.2-0.7) in the Netherlands case-control study. The results suggest that this may be especially important for women consuming a diet high in red meat (Chapter 7).

Since prospective studies are less prone to biases related to the presence of disease or selection of subjects, the association between fermented dairy products, dietary calcium and colorectal cancer was additionally studied in The Netherlands Cohort Study on diet and cancer (Chapter 8). This prospective study started in 1986 when 120,852 Dutch men and women, aged 55-69, completed a food frequency questionnaire. The case-subcohort analysis was based on 3.3 years of follow-up and included 215 incident cases of colon cancer and 111 incident cases of rectal cancer, excluding cases diagnosed in the first year of follow-up. Colorectal cancer risk was not significantly related to the consumption of fermented milk (RR = 0.9, 95% CI = 0.6-1.3) and cheese (RR = 0.9, 95% CI = 0.6-1.3).

Dietary calcium intake was not significantly associated with risk either (RR = 0.9, 95% CI = 0.6-1.3). Only calcium from unfermented dairy products was inversely associated with rectal cancer risk (RR = 0.6, 95% CI = 0.3-1.0, trend p = 0.03).

Chapter 9 summarizes the results and discusses the strengths and limitations of these four studies. Results are discussed in the context of the findings of previous epidemiological studies and experimental research. Overall, the results of the four studies described in this thesis do not support the hypothesized preventive potential of fermented dairy products and dietary calcium in colorectal carcinogenesis. These findings contrast with previous case-control studies addressing fermented milk (yogurt and buttermilk), and strengthen the null findings of 12 out of 21 epidemiological studies that addressed dietary calcium. The null findings of our studies are not likely to be explained by the end-points studied, the methods of exposure assessment, the variation in fermented dairy product consumption or calcium intake, the actual level of calcium intake, or confounding. However, these studies do not exclude the possibility that inverse associations might still be found for dairy products cultured with other bacterial strains.

Further epidemiological studies on fermented dairy products, calcium and colorectal cancer should especially explore different sites within the colon, preferably including markers of genetic susceptibility. Epidemiological as well as experimental research should focus more explicitly on different end-points, sex differences and the effects of commercially available fermented dairy products in the aetiology of colorectal cancer.

Our findings of a neutral effect of dairy products and, as observed in the case-control study, a protective effect of vegetables especially in women consuming a high-meat diet (Chapter 7), are in line with the dietary recommendations put forward by the Dutch Nutrition Council and the Dutch Cancer Society.

Samenvatting

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Dikke darmkanker (colon- en rectumkanker) is één van de belangrijkste vormen van kanker in westerse landen; in Nederland worden ieder jaar ongeveer 7000 nieuwe gevallen gediagnostiseerd. Hoewel de vijf-jaars overleving stijgt bij vroege diagnostiek, wordt geen belangrijke vooruitgang op therapie-gebied verwacht in de nabije toekomst. Preventie van deze vorm van kanker blijft daarom van groot belang.

In hoofdstuk 2 wordt een overzicht gegeven van de huidige kennis over de oorzaken van dikke darmkanker. In de afgelopen 20 jaar zijn verschillende fasen in de vorming van darmtumoren geïdentificeerd en is gebleken dat darmkanker ontstaat door verschillende mutaties in oncogenen en tumorsuppressorgenen. De meeste darmtumoren ontstaan uit adenomateuze poliepen. Zowel genetische factoren als omgevingsfactoren kunnen het risico op het ontstaan van een darmpoliep of tumor beïnvloeden. Wat betreft omgevingsfactoren wordt voeding als één van de belangrijkste risicofactoren beschouwd, aangezien de darmwand in direct contact staat met voedselbestanddelen en metabolieten hiervan. Een algemeen geaccepteerde hypothese betreft een verhoging van het darmkankerrisico door een westers voedingspatroon dat gekenmerkt wordt door een hoge vet- en een lage vezel-inneming. Wat voedingsmiddelen betreft lijkt vleesconsumptie het risico te verhogen, terwijl de consumptie van groente en fruit het risico zou kunnen verlagen. Naast een toename van de groente- en fruitconsumptie is verondersteld dat een toename van de consumptie van gefermenteerde zuivelprodukten, zoals yoghurt, karnemelk en kaas, eveneens een bijdrage zou kunnen leveren aan de preventie van dikke darmkanker. Experimentele studies suggereren dat een eventuele bescher-mende werking van gefermenteerde zuivelprodukten toegeschreven zou kunnen worden aan de Lactobacillus bacteriën die deze produkten bevatten of aan het relatief hoge calcium gehalte van zuivelprodukten in het algemeen. Bepaalde Lactobacillus stammen kunnen de normale bacterieflora in de dikke darm gunstig beïnvloeden, het immuunsysteem stimuleren en/of mutagene stoffen binden. Calcium zou de deling van darmwandcellen indirect kunnen beïnvloeden door toxische secundaire galzuren en vrije vetzuren te binden of direct door cel-differentiatie te induceren. Tot nu toe is slechts in een beperkt aantal epidemiologische studies de relatie tussen de consumptie van gefermenteerde zuivelprodukten en het ontstaan van darmkanker onderzocht. Meerdere studies zijn uitgevoerd naar de relatie met calciuminneming, maar de resultaten van deze studies lijken niet consistent te zijn.

Dit proefschrift beschrijft en bediscussieert de resultaten van vier epidemiologische studies waarin is onderzocht of het gebruik van gefermenteerde zuivelprodukten en de inneming van calcium invers geassocieerd zijn met het ontstaan van dikke darmkanker. In deze studies zijn verschillende eindpunten van de ziekte bestudeerd (adenomateuze poliepen en tumoren) en zijn verschillende epidemiologische onderzoeksmethoden (retrospectief en prospectief) en verschillende methoden van voedselconsumptie onderzoek gebruikt (dietary history en schriftelijke voedselfrequentie vragenlijsten). Tevens zijn de studies uitgevoerd in verschillende populaties (Amerika, Nederland, mannen en vrouwen) van verschillende leeftijd en met een verschillend consumptiepatroon van gefermenteerde zuivel.

Hoofdstuk 3 beschrijft de resultaten van twee van deze studies: de Nurses' Health Study (NHS: 98.464 vrouwen, 30-55 jaar aan het begin van de studie) en de Health Professionals Follow-up Study (HPFS: 51.529 mannen, 40-75 jaar aan het begin). Deze prospectieve studies worden momenteel uitgevoerd aan de Harvard School of Public Health in Boston, Verenigde Staten. De associatie tussen de consumptie van gefermenteerde zuivelprodukten, de inneming van calcium en het voorkomen van adenomateuze darmpoliepen is bestudeerd gebruikmakend van een semi-quantitatieve schriftelijke voedselfrequentievragenlijst. Het voedingspatroon van patiënten (331 mannen en 350 vrouwen) met adenomateuze poliepen in het linker colon en het rectum gediagnostiseerd binnen vier (NHS en HPFS) of acht jaar (NHS) na het invullen van de vragenlijst, zijn vergeleken met het patroon van personen bij wie tijdens endoscopie géén adenomen werden waargenomen (9.159 mannen en 9.858 vrouwen). Na correctie voor leeftijd, totale energie-inneming, het voorkomen van dikke darmkanker in de familie, en de inneming van verzadigd vet, was de consumptie van gefermenteerde zuivelprodukten niet geassocieerd met het ontstaan van darmpoliepen (NHS: relatief risico hoogste versus laagste consumptiecategorie (RR) = 0,8; 95% betrouwbaarheidsinterval (btbhi) = 0,5-1,1); HPFS: RR = 1,1; 95% btbhi = 0,7-1,6). Correctie voor andere mogelijk verstorende variabelen veranderde deze resultaten nauwelijks. De calciuminneming uit de voeding was evenmin geassocieerd met ontstaan van poliepen, zowel voor mannen (multivariate RR hoogste versus laagste quintiel van inneming = 1,1; 95% btbhi = 0,8-1,7) als vrouwen (RR = 1,2; 95% btbhi = 0,8-1,7). Er werden geen belangrijke verschillen in RRs gevonden voor calcium uit de voeding of uit supplementen. Hoewel geen associatie met vitamine D uit de voeding werd gevonden, was vitamine D uit supplementen, hoewel niet significant, invers geassocieerd met het ontstaan van poliepen bij vrouwen (RR = 0,7; 95% btbhi = 0,4-1,1).

Het neutrale effect van zuivelprodukten en, zoals gevonden in het patiënt-controle onderzoek, het beschermend effect van groente (speciaal voor vrouwen met een relatief hoge vleesconsumptie) ondersteunen de voedingsaanbevelingen van de Nederlandse Kankerbestrijding en de richtlijnen goede voeding van de Nederlandse Voedingsraad.

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About the author

Ellen Kampman was born in Utrecht, the Netherlands, on April 29th 1962. After secondary school (Athenaeum B) at 'Schoonoord' in Zeist, she studied Human Nutrition at the Department of Nutrition, Agricultural University, Wageningen. The M.Sc. program focused primarily on epidemiology, human nutrition and toxicology. She graduated with honours in June 1988, after a five months training period at the Athens School of Public Health in Athens, Greece.

Subsequently, she was appointed as part-time research associate at the Department of Human Nutrition, Agricultural University in Wageningen and as part-time tumor registrar at the Comprehensive Cancer Centre Middle Netherlands in Utrecht.

In November 1989, she started the research described in this thesis at the Department of Nutrition, TNO Nutrition and Food Research in Zeist. As part of this research, she worked at the Departments of Nutrition and Epidemiology at the Harvard School of Public Health, Boston, US, in the academic year 1991 for a period of six months. During her stay in Boston, she attended the New England Epidemiology Summer Program and courses in nutritional epidemiology and statistics at the Harvard School of Public Health.

She attended a course on 'molecular biology for cancer epidemiologists' organized by the Internal Agency for Research on Cancer in Lyon, France, was further trained in molecular biology during her stay at Harvard and was recently rewarded with a two-year epidemiologic fellowship from the Netherlands Cancer Foundation to specialize in molecular epidemiology. Experience will be gained with the implementation of molecular tools in epidemiologic studies at the Cancer Prevention Program of the Fred Hutchinson Cancer Research Center, Seattle, US. The Department of Epidemiology and Public Health at the Agricultural University of Wageningen will be the home address for the fellowship in the Netherlands.

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Stellingen

1. Yoghurt, karnemelk of kaas lijken bij dagelijks gebruik niet te beschermen tegen darmkanker (dit proefschrift).

2. Net als in de VS is in Nederland het gebruik van yoghurt en karnemelk geassocieerd met een gezond leefpatroon (dit proefschrift).

3. Dat vooral vrouwen een voorkeur hebben voor zure produkten (dit proefschrift) verklaart nog niet hun hogere levensverwachting.

4. De waarneming dat het krijgen van kinderen bij de man niet, en bij de vrouw wél beschermt tegen darmkanker (dit proefschrift) zou behalve door hormonale factoren ook te verklaren kunnen zijn door de achterblijvende emancipatie van de Nederlandse man.

5. Het gebruik van de kankerregistratie halveert de periode nodig voor patiëntenwerving en verdubbelt de kwaliteit van de gegevens (dit proefschrift).

6. Alvorens patiënt-gebonden onderzoek uit te voeren zouden de onderzoekers zich voor beperkte tijd chronisch ziek moeten voelen.

7. Wetenschap is soms als modern theater: de vorm overheerst dan de inhoud.

8. Als zwijgen goud is, dan bestaat er nog een kans voor de minderheden in deze wereld.

9. Het bevorderen van groente- en fruitconsumptie verhoogt de noodzaak tot gescheiden inzameling van afvalstoffen.

10. There are different levels of explanation that cast light, each upon the others. (Potter JD. Reconciling the epidemiology, physiology, and molecular biology of colon cancer. JAMA 1992;268:1573-77).

11. Verlegenheid is niet de enige determinant van het blozen.

Stellingen behorend bij het proefschrift `Do fermented dairy products and calcium protect against colorectal cancer? Contributions from epidemiology'
Ellen Kampman, 30 september 1994