Barrett's esophagus and esophageal and gastric cancer subtypes : an epidemiologic perspective

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an epidemiologic perspective

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1

General introduction

Cancer has become a disease that affects many people in the world. The International Agency for Research on Cancer estimated that 12.7 million new cancer cases and 7.6 million cancer deaths occurred in 2008.¹ In the Netherlands, cancer has become the most important cause of death in 2008.² In that year, over 40,000 persons died of cancer, which was approximately 30% of all deaths.³

This thesis is concerned with two types of cancer: cancer of the esophagus and cancer of the stomach, also called gastric cancer. Within esophageal and gastric cancer one can discern subtypes. Besides these cancers, this thesis also examines Barrett's esophagus (BE), a precursor of adenocarcinoma of the esophagus.

ESOPHAGEAL AND GASTRIC CANCER

Incidence and mortality

Esophageal cancer was estimated to be the 8th most common cancer worldwide with regard to new cases, and the 6th most common cause of death from cancer in 2008. Cancer of the stomach is more common being the 4th cancer with regard to new cases, and the 2nd leading cause of cancer death in the world.¹

Geographically, esophageal and gastric cancer are not distributed evenly (Figures 1.1 and 1.2). Esophageal cancer is specifically common in Asia (Iran, China, Japan, Korea), Southern and Eastern Africa (Uganda, Zimbabwe), USA (Blacks), and France. Typically, in these high risk populations the majority of esophageal cancers is of the squamous cell type, in contrast to low risk populations, where adenocarcinomas are more common.⁴ Incidence rates for gastric cancer are highest in Eastern Asia (China, Japan, Korea), Eastern Europe, Italy, Portugal and Latin America.^{1,5} In the Netherlands, 1876 and 2020 persons were diagnosed with esophageal and gastric cancer, respectively, and 1558 and 1424 patients died of these cancers in 2008.⁶

Course of the diseases

Symptoms that indicate the presence of esophageal cancer are dysphagia, weight loss, and pain on swallowing foods.⁸ The most common symptoms that precede a diagnosis of gastric cancer are weight loss, abdominal pain, nausea, and dysphagia.⁹ Patients are often not diagnosed with esophageal or gastric cancer until the cancer has reached an advanced stage. Partly due to this late diagnosis, treatment options are limited and curative treatments are often not possible.¹⁰ The late diagnosis therefore lies at the bottom of the very high mortality rate of both cancers. One year after diagnosis, 42% of the esophageal cancer patients and 45% of the gastric cancer patients are still alive. Only 14% of esophageal and 21% of gastric cancer patients survive five years.⁶ These Dutch rates are comparable with European survival rates.¹¹

Figure 1.1 Estimated worldwide incidence rates (per 100,000) of esophageal cancer (both sexes, all ages), age-standardized to the world population. Source: Globocan 2008.⁷

Figure 1.2 Estimated worldwide incidence rates (per 100,000) of gastric cancer (both sexes, all ages), age-standardized to the world population. Source: Globocan 2008.⁷

Classification of esophageal and gastric cancers

Cancers of the esophagus and stomach can be classified in several ways. In the International Classification of Diseases for Oncology (ICD-O),¹² tumors are classified according to their topography and histology.

For the esophagus, there are two possible topographic classifications: cervical, thoracic, and abdominal part of the esophagus, or upper, middle, and lower third of the esophagus. The latter classification is most commonly used in research, and shown in Figure 1.3. Another classification is based on the type of tissue from which the tumor originates: a histological classification. The two most common histological types of esophageal cancer are esophageal squamous cell carcinoma and esophageal adenocarcinoma. Esophageal squamous cell carcinoma most often occurs in the middle third of the esophagus, while the large majority of esophageal adenocarcinomas arise in the lower third.¹³

The ICD-O topographic classification of gastric cancer discerns tumors of the cardia, fundus, body, pyloric antrum, and pylorus (Figure 1.3). Additionally, tumors can be classified as located in the lesser or greater curvature of the stomach.¹² The large majority of all gastric cancers are adenocarcinomas.¹³

Figure 1.3 The esophagus and stomach.

Trends in incidence rates

Incidence rates of esophageal and gastric cancer have been carefully monitored for decades. Before 1940, gastric cancer was the leading cause of cancer death in men in the USA. In the next decades, however, this cancer became less and less common. Between 1950 and the 1970s, gastric cancer mortality had started to decline quite sharply in several countries, including the USA, Scandinavian countries, Western European countries, and Australia.^{4,14} Later publications observed, however, that this decline in gastric cancer did not concern the cardia, the proximal part of the stomach.¹⁵ In fact, some reported that the incidence of gastric cardia cancer had risen in the 1960s through 1980s in the USA, the UK, Denmark, and Sweden.¹⁶⁻¹⁹ Moreover, these and other studies also observed sharp increases in the incidence of adenocarcinomas of the esophagus.^{16-18,20} In contrast, stable or declining incidence rates have been reported for the other histological type of esophageal cancer: squamous cell carcinoma.^{16,17,21-23}

The question continually is, however, whether these observed changes in incidence are true increases in the disease. In the course of time the attention for subtypes of esophageal and gastric cancer has grown, which may have influenced clinicians and pathologists. Also, the quality of registration of tumor histology and subsite has improved greatly.^{5, 24-28} These developments may have contributed to the observed changes. In general, it is thought that at least a part of the observed increase in adenocarcinomas of the esophagus and gastric cardia and decrease in other gastric adenocarcinomas is real.^{16,19,20,22,23}

As a result of the trends in incidence rates, the ratio of esophageal squamous cell carcinoma (ESCC) to esophageal adenocarcinoma (EAC) has changed in USA whites from 83/17 in 1974 to 41/59 in 1995.²⁹ In contrast, in USA blacks the ratio remained stable at 97/3 during this period.²⁹ In the Netherlands this ratio changed from 55/45 to 34/66 between 1989 and 2003.⁶ Thus, in the USA and the Netherlands, EAC has become the most common histological type of esophageal cancer. Conversely, this was not observed in China, a high risk area of esophageal cancer, where ESCC accounted for nearly 100% of esophageal cancers, at least in the period 1988-2003.³⁰ The subsite distribution of gastric cancers has also changed over time as a consequence of the incidence trends. In the USA, the percentage GCA of all gastric tumors increased from 8% to 15% in black men and from 30% to 47% in white men between 1974 and 1995.²⁹ In contrast, in the Netherlands this percentage remained stable around 25% between 1990 and 2007.³¹ A relatively high percentage of GCA tumors is seen in China: in 1993 GCA cases accounted for 30% of gastric cancers, and this increased to 37% in 2004.³² Thus, the geographical differences in the percentage of GCA cases are less striking than the differences in the ratio of ESCC to EAC.

In the future, it remains important to keep monitoring trends in the incidence of esophageal and gastric cancer subtypes. High quality and detailed data are required for this monitoring. Interpretation of these trends should be done carefully by taking into account changes in diagnosis and registration of these cancers. A further aim is to

explain the observed trends and to identify risk factors for these diseases. Primary prevention is specifically important given the poor prognosis, as well as identification of high-risk individuals and early detection (secondary prevention) of esophageal and gastric cancer.

BARRETT'S ESOPHAGUS

Barrett's esophagus (BE), also referred to as columnar lined esophagus, is a so-called precancerous lesion for esophageal adenocarcinoma. In persons with BE, the normal multi-layer squamous epithelium of the esophagus is replaced by a single layer of columnar epithelium. When a patient is undergoing endoscopy of the esophagus, this metaplasia is visible. The columnar epithelium has a red color as opposed to the light pink color of the normal squamous epithelium (Figure 1.4).³³ BE is located in the lower third of the esophagus.

Figure 1.4 Endoscopic picture of Barrett's esophagus. Arrows indicate columnar epithelium. Source: ³⁹

Definition and diagnosis of the disease

The condition BE was named after Norman Barrett, a surgeon who described it in 1950.³⁴ The definition of BE has been debated and has changed over time.^{33,35} Currently there are two different accepted case definitions. The American College of Gastroenterology definition is as follows: "Barrett's esophagus is a change in the distal esophageal epithelium of any length that can be recognized as columnar type mucosa at endoscopy and is confirmed to have intestinal metaplasia by biopsy of the tubular esophagus."³⁶ The British Society of Gastroenterology defines Barrett's esophagus as "an oesophagus in which any portion of the normal squamous lining has been replaced

by a metaplastic columnar epithelium which is visible macroscopically. In order to make a positive diagnosis of “Barrett’s oesophagus”, a segment of columnar metaplasia of any length must be visible endoscopically above the oesophago-gastric junction and confirmed or corroborated histologically.”^{37,38} Thus, in contrast to the American definition, the British definition does not require identification of specialized intestinal metaplasia. The British Society of Gastroenterology reasons that intestinal metaplasia can always be found, providing a sufficient number of biopsies is taken over an adequate time-scale.³⁷

Incidence rates

After it was observed that the incidence of esophageal adenocarcinoma was rising in various countries, researchers hypothesized that this increase was preceded by a similar rise in the incidence of BE. Substantial increases in diagnosis of BE have indeed been described in Scotland, the USA, and the Netherlands.⁴⁰⁻⁴²

In the Netherlands, the world standardized incidences rates of BE increased from 12.7 to 18.4 per 100,000 men and from 6.5 to 8.6 per 100,000 women between 1992 and 2003. This increase is not explained by an increase in the number of biopsies taken, or by an increased proportion of BE patients that undergo endoscopy. Therefore, a real increase in the incidence of BE seems likely.⁴²

Progression and surveillance

As mentioned earlier, BE is a precancerous lesion, or precursor lesion. For three decades, it has been known that patients diagnosed with BE are at increased risk of esophageal adenocarcinoma.⁴³ However, the exact size of this risk remains uncertain. The risk of progression has been estimated by several researchers and the reported risk estimates are highly variable, depending on the study population, selection of patients, and follow-up time.⁴⁴ Large studies with complete follow-up that quantify this risk are requested. Another question that remains to be answered is whether patients with BE are maybe also at increased risk of other cancers and other diseases.

The progression of BE can be identified pathologically. The successive steps are BE without dysplasia, low-grade dysplasia, high-grade dysplasia and finally adenocarcinoma (Figure 1.1).⁴⁵

Studying BE is interesting and important, because it may yield new insights into the etiology of the disease, which is important for primary prevention. Ultimately, research should lead to the identification of persons at highest risk of developing esophageal adenocarcinoma. Through endoscopic surveillance of patients with BE, esophageal adenocarcinoma may be diagnosed in an earlier stage. This may lead to improved treatment options and prognosis.

RELEVANCE OF CANCER SUBTYPES AND PRECURSOR LESIONS

So far, we described two indications for a different etiology of esophageal squamous cell carcinoma (ESCC) vs. esophageal adenocarcinoma (EAC), and gastric cardia adenocarcinoma (GCA) vs. gastric non-cardia adenocarcinoma (GNCA). These are the differences in trends in incidence rates and the differences in the geographical distribution of these cancer subtypes.

A third indication for a different etiology is the observed sex difference in the occurrence of these cancers. All subtypes of esophageal and gastric cancer occur more frequently in men than women, at least in most parts of the world.¹³ But more importantly, the ratio of the incidence in men to that in women, the male-to-female ratio, is much higher for EAC (between 6:1 and 8:1),⁴⁶⁻⁴⁸ compared with ESCC (between 2:1 and 3:1).^{46,49} For BE, the male-to-female ratio is usually around 2:1.⁵⁰ A different male-to-female ratio is also observed for the gastric cancer subtypes. The ratio is between 3:1 and 5:1 for GCA,^{46,48,51,52} whereas it is around 2:1 for GNCA.^{18,51}

Until recently, relatively little attention was directed towards differences between the histologic types of esophageal cancer and the majority of existing epidemiologic studies have not distinguished between the major histologic types of esophageal cancer. This also applies to the subsite division of gastric cancer.

BE has started to receive more attention since a rise in incidence of EAC has been observed in several countries. BE is an interesting precursor lesion, because it is very well visible through endoscopy and can thus be monitored and studied relatively easily. It thus provides a good model for the investigation of cancer development. One thing we should keep in mind is that BE is only diagnosed if a person presents with reflux symptoms or if endoscopy is performed on different medical grounds and BE is found by chance. Thus, some cases of asymptomatic BE remain undetected. The estimates of the proportion of asymptomatic individuals that have BE vary widely: between 1 and 25%.⁵³⁻⁵⁵

Precancerous lesions have also been identified for ESCC,⁵⁶ GCA,⁵⁷ and GNCA.⁵⁸ These are, however, not the focus of this thesis.

RISK FACTORS FOR ESOPHAGEAL AND GASTRIC CANCERS AND BARRETT'S ESOPHAGUS

Demographic factors

As with nearly all cancers, older age is a strong risk factor for esophageal and gastric cancers,¹³ as well as for BE.⁵⁹ As described above, men are at higher risk of all esophageal and gastric cancer subtypes and BE than women. In the USA, BE and EAC are more common among Caucasians, while ESCC is more common among blacks.¹³

Medical factors

Several medical conditions and medications have been associated with risk of BE and esophageal and gastric cancer. The most important risk factor for BE and EAC is gastro-esophageal reflux disease (GERD),⁶⁰ which is defined as chronic symptoms or mucosal damage produced by the abnormal reflux of gastric contents (including acid) into the esophagus.⁶¹ GERD may also increase risk of GCA, although probably less strongly than for BE and EAC.⁶² Gastro-esophageal reflux can be increased in the presence of a hiatal hernia, which means that the upper part of the stomach protrudes into the chest cavity through a hole in the diaphragm.¹³ Not only reflux of acid can increase risk of esophageal cancer: ingestion of caustic substances, particularly lye, very strongly increase the risk of ESCC.⁶³

ESCC risk is also higher in subjects with achalasia and tylosis. Achalasia is an esophageal motility disorder involving the smooth muscle layer of the esophagus and the lower esophageal sphincter (LES). Patients with achalasia have difficulty swallowing and suffer from regurgitation.⁶³ Tylosis is a rare disease associated with hyperkeratosis of the palms of the hands and soles of the feet. The inherited type of tylosis (Howell-Evans syndrome) has been most strongly linked to ESCC.⁶³

There is also evidence for a relationship between some medications and risk of BE or esophageal or gastric cancer. Medications that relax the LES may contribute to increased risk of BE and EAC via increased chance of gastro-esophageal reflux. These medications include the commonly used nitroglycerin, aminophyllines, beta-adrenergic agonists, anticholinergics and benzodiazepines.^{64, 65} Nonsteroidal anti-inflammatory drugs (NSAIDs) on the other hand, may be preventive for EAC and maybe also for ESCC and GNCA,^{13,66-68} possibly because they stimulate apoptosis and inhibit angiogenesis.⁶⁶

Infection with *Helicobacter* (H.) *pylori* has been identified as the strongest risk factor for gastric cancer.¹³ However, this is probably only true for GNCA. In contrast, H. *pylori* appears to protect against EAC and GCA.^{13,47} Infection with Epstein-Barr virus has also been suggested to play a role in some gastric tumors.¹³ Other medical conditions that have been suggested to increase risk of gastric cancer include pernicious anemia, peptic ulcer disease, and prior gastric surgery.⁵² Further, persons with a family history of gastric cancer have been observed to have an increased risk to develop gastric cancer.¹³

Lifestyle and dietary habits

Several aspects of lifestyle have been studied in relation to risk of developing esophageal and gastric cancer. Most studies have not separately studied the cancer subtypes. Very little information is available on possible relationships between lifestyle and diet and the risk to develop BE.

Smoking and alcohol consumption have been studied most, and have been found to increase risk of ESCC. Smoking is also associated with EAC and gastric cancers, but probably less strongly. The association between alcohol consumption and risk of EAC

and gastric cancers is not entirely clear yet.¹³ Further, being overweight may increase a person's risk of EAC and GCA.^{13, 69}

Higher incidence rates of ESCC and gastric cancer have been observed among the lower socioeconomic groups. This observation may be explained partly by risk factors strongly associated with low socioeconomic status (SES), such as infection with *H. pylori* and smoking. On the other hand, the association with SES may also reflect yet unrecognized exposures, as the association between SES and ESCC risk remained after adjustment for known risk factors.¹³

Vegetables, fruits, and some vitamins and carotenoids may decrease risk of esophageal and gastric cancer. Foods containing dietary fiber may decrease risk of esophageal cancer. On the other hand, there is some indication for a positive association between processed meat and esophageal and gastric cancer. Esophageal cancer risk may also be increased by eating red meat and drinking maté and very hot drinks. Salt, salted foods, chili, smoked foods, and grilled or barbecued animal food may increase gastric cancer risk.⁶⁹

RATIONALE AND AIM OF THIS THESIS

As explained in the previous paragraphs, there has been little epidemiologic research into the risk factors for subtypes of esophageal and gastric cancer. The data on BE is even more limited. Very few studies have looked into the etiology of this condition. Besides, most evidence on BE and esophageal and gastric cancer subtypes to date comes from cross-sectional and case-control studies. These study designs are susceptible to methodological limitations. The prospective cohort study design is less susceptible to biases and therefore generally has a higher validity. The research described in this thesis is carried out within a prospective cohort study: the Netherlands Cohort Study on diet and cancer (NLCS).

Our study could make an important contribution to the epidemiological body of evidence on the development of BE and the subtypes of esophageal and gastric cancer. The cancers of main interest in this thesis are ESCC, EAC, and GCA. GNCA will be described only for comparison with these cancers. The main focus of this thesis is on the role of lifestyle and nutritional factors in the etiology of these diseases. These factors can potentially be modified and can thus have a role in primary prevention.

Additionally, we will use the data of the NLCS to follow-up BE patients for occurrence of EAC, other cancers and cause-specific mortality, as we indicated that data are lacking on these topics. The large size and complete follow-up make the NLCS suitable for these analyses.

Finally, we will also study changes in the incidence of esophageal and gastric cancers in time, because of the need to keep monitoring trends for above-mentioned reasons. We will do this with data from European countries, and examine whether there are differences in trends between countries.

STUDY DESIGN

The research described in this thesis is based on a prospective cohort study: the Netherlands Cohort Study on diet and cancer (NLCS). The NLCS was initiated in September 1986.⁷⁰ A total of 120,852 men and women aged 55-69 years participated in this study. These subjects were randomly selected from Dutch municipal population registries. At baseline, all subjects filled out a questionnaire on dietary habits, lifestyle, and other risk factors for cancer. According to the case-cohort approach,⁷¹ data are processed and analyzed for a random sample of the cohort (the subcohort) and cases. The subcohort consists of 5,000 men and women who were sampled at baseline. This subcohort is followed-up for vital status and migration and is used to estimate the person-time at risk for the total cohort. Incident cases of BE and cancer were identified in the whole cohort. The identification of BE cases took place through linkage with data from PALGA (the nationwide registry for histopathology and cytopathology in the Netherlands). The existence of this unique nationwide pathology registry offered us the opportunity to study BE within a prospective cohort study. A pathologist reviewed and coded further details and characteristics of the BE cases from the PALGA data. The cohort was also followed-up for cancer incidence, and this was done through linkage with PALGA and the Netherlands Cancer Registry.⁷² Further, the cohort has been followed-up for vital status through record linkage with the Central Bureau of Genealogy and automated municipal population registries. For deceased cohort members we also obtained information on the cause of death from Statistics Netherlands.

The research described in this thesis was based on 16.3-year follow-up data of the cohort (September 17, 1986 through December 31, 2002).

OUTLINE OF THE THESIS

This thesis begins with two studies on the etiology of BE. Chapter 2 describes the associations between the lifestyle factors overweight, smoking, and alcohol consumption and the risk to develop BE. Our study on selenium status and its relationship with risk of BE is presented in chapter 3. Chapter 4 focuses on the question whether cancer incidence and total and cause-specific mortality are increased in patients with BE. Trends in the incidence of esophageal and gastric cancer subtypes in European countries are investigated in chapter 5. The next three chapters are about several possible risk factors of esophageal and gastric cancer subtypes: alcohol consumption and cigarette smoking (chapter 6); selenium status (chapter 7), and vegetables and fruits consumption (chapter 8). Finally, chapter 9 concludes the thesis with a discussion of the main findings and some methodological aspects.

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2

A prospective cohort study on overweight, smoking, alcohol consumption, and risk of Barrett's esophagus

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ABSTRACT

Background

Barrett's esophagus (BE) is a precursor lesion of esophageal adenocarcinoma. Besides gastroesophageal reflux, possible risk factors for BE include overweight, cigarette smoking, and alcohol consumption. Our objective was to study these associations using prospective data.

Methods

The prospective Netherlands Cohort Study, initiated in 1986, consists of 120,852 men and women, aged 55-69 years at baseline. At baseline, all subjects completed a questionnaire on dietary habits and lifestyle. After 16.3 years of follow-up, 370 BE cases with specialized intestinal metaplasia and 3866 subcohort members were available for case-cohort analysis. Cox proportional hazards models were used to calculate incidence rate ratios (RR) and 95% confidence intervals (CI).

Results

Body mass index at baseline was associated with risk of BE in women [multivariable adjusted RR per 1 kg/m², 1.07 (1.03-1.11)] but not in men [RR per 1 kg/m², 0.99 (0.93-1.05)]. The association in women was not specifically due to abdominal overweight. Former cigarette smokers were at increased risk of BE (RR=1.33, 95% CI 1.00-1.77), but current smokers were not. Smoking duration showed a positive association with BE risk (p trend=0.03). For alcohol consumption, the RR per 10 grams ethanol/day was 0.95 (0.87-1.03).

Conclusions

Increased body mass index was a risk factor for BE in women, but not in men. Several aspects of cigarette smoking were positively associated with BE risk. Alcohol consumption was not associated with an increased risk of BE.

Impact

Future research should focus on risk factors for development as well as for progression of BE to esophageal adenocarcinoma.

INTRODUCTION

Barrett's esophagus (BE) is a condition of the distal esophagus characterized by a replacement of the normal stratified squamous epithelium with a single layer of columnar epithelium. Histological confirmation based on biopsies is required for diagnosis. According to the USA definition of BE the presence of goblet cells, indicating specialized intestinal metaplasia (SIM), is required.¹ The UK definition is less stringent: the presence of any type of metaplasia is sufficient for a diagnosis of BE.² Both the USA and UK definitions may have been used in the Netherlands.

BE is primarily of interest because it is associated with an increased risk of esophageal adenocarcinoma, a cancer with high mortality. The estimates of this risk of esophageal adenocarcinoma vary between 4.1 and 6.1 per 1000 person-years.³

The most recognized and strong risk factor for BE is chronic gastroesophageal reflux.⁴ Additionally, overweight, cigarette smoking, and alcohol consumption might be associated with risk of BE. These factors are interesting as they are potentially modifiable. However, the body of evidence on the role of these factors in the etiology of BE is sparse. Especially data from prospective cohort studies is lacking.

The possible role of overweight in the etiology of BE has been investigated in several epidemiological studies. Null associations^{5,6} as well as positive associations⁷⁻⁹ have been found in case-control studies. The only prospective cohort study, which was conducted among women, reported a positive association.¹⁰ The relationship between cigarette smoking and risk of BE has been studied in some case-control studies,^{6,8,11,12} while no cohort studies have reported on this relationship. Some observed a positive association,^{6,8} while other studies did not observe an association.^{5,12} Alcohol consumption and its possible relationship with BE has been studied in a few case-control studies. Null-associations with alcohol have been observed,^{11,13} and one study¹⁴ found alcohol to be a risk factor. Inverse associations have been reported for wine consumption.^{11,13}

The aim of this study was to investigate the associations between overweight, smoking, and alcohol consumption and risk of BE, within the prospective Netherlands Cohort Study (NLCS) on diet and cancer.

METHODS

Study design and subjects

The NLCS was started in September 1986. Cohort members were selected at random from 204 Dutch municipal registries, and 58 279 men and 63 573 women aged 55-69 were enrolled in the study. All members of the cohort completed a self-administered questionnaire. A detailed study design has previously been published.¹⁵

For efficiency, we used a case-cohort approach¹⁶ for data processing and analysis. Cases were derived from the entire cohort, while the number of person-years at risk in the entire cohort was estimated from a subcohort of 5000 subjects. This subcohort was selected at random from the full cohort at baseline.

The subcohort was followed-up for vital status, first actively by biennially contacting the subcohort members, and later by linkage to the Dutch municipal population registries. After 16.3 years (September 1986 to December 2002), only one male subcohort member was lost to follow-up. We excluded subcohort members who reported having prevalent BE or cancer (other than skin cancer) (n=230) at baseline (Figure 2.1).

The Medical Ethics Committee of Maastricht University, the Netherlands, has approved the study.

Follow-up

Incident BE cases in the total cohort were detected by computerized record linkage to the nationwide network and registry of histopathology and cytopathology in the Netherlands (PALGA).¹⁷ This network was founded in 1971, and an increasing number of laboratories joined PALGA such that it covered all 204 municipalities from which the cohort members were sampled since 1991. At each of the 64 pathology laboratories in the Netherlands, summaries of all pathology reports are generated automatically. Each summary contains a so-called PALGA diagnosis that describes topography, morphology, function, procedure, and disease. These excerpts are transferred to a central databank.¹⁷ The linkage with PALGA was carried out for 16.3 years of follow-up.

Subsequently, one pathologist (A.D.) and one pathologist in training (C.H.), who were blinded to the exposure status of the cases, reviewed the excerpts of all pathology records. Excluded were cases with an uncertain diagnosis of BE (n=106) and cases that had prevalent cancer or BE at baseline (n=76). Additionally, we excluded cases with a diagnosis of esophageal or gastric cancer before or less than a half year after the diagnosis of BE (n=58), and cases with a diagnosis specifying the presence of only non-intestinal type metaplasia (n=108) (Figure 2.1).

Two definitions of BE were used: our primary case definition included only subjects with esophageal SIM (n=456). The secondary case definition included subjects (a) fulfilling the primary case definition or (b) with a pathology report stating 'Barrett's', without a description of the type of metaplasia (total n=626).

Exposure data

All cohort members completed a self-administered questionnaire at baseline. This questionnaire contained a 150-item food frequency questionnaire (FFQ), including detailed questions on alcohol consumption, and questions on various other cancer risk factors.

Overweight was measured by several variables: BMI at baseline, BMI at age 20 years, BMI change, and pant/skirt size (as a proxy for waist circumference¹⁸). BMI at baseline and BMI at age 20 years were calculated using weight at baseline and weight at age 20 years, respectively, divided by height at baseline squared (kg/m^2). Subjects with missing values for BMI at baseline were excluded from all analyses. Subjects with a BMI under 18.5 were excluded, because there were no cases in this category. BMI change since age 20 years was calculated as BMI at baseline minus BMI at age 20 years. Recently, the use of self-reported pant/skirt size as a proxy measure for waist circumference was validated in the NLCS.¹⁸

Questions were asked about the following aspects of cigarette smoking: whether the subject was a smoker at baseline, age at smoking initiation, age at smoking cessation, the number of cigarettes smoked daily and the number of smoking years (excluding stopping periods). Based on these questions, the following variables were constructed: smoking status (never/former/current), current smoking (yes/no), frequency (n cigarettes/day), duration (n years), pack-years of cigarette smoking (n) and time since cessation (years).

The habitual consumption of alcohol during the year preceding the start of the study was measured by six items in the questionnaire: (a) beer, (b) red wine, (c) white wine, (d) sherry and other fortified wines, (e) liquor types containing on average 16% alcohol and (f) (Dutch) gin, brandy and whiskey. Questions were asked about the frequency of consumption and the number of glasses consumed on each drinking occasion. For analysis, we combined (b), (c) and (d) into 'wine', and (e) and (f) into 'liquor'. Mean daily alcohol consumption was calculated using the Dutch food-composition table.¹⁹ For 'beer' and 'other alcoholic beverages', participants could indicate whether five years ago, they drank (a) more than, (b) equal amounts of or (c) less than today. The fourth answering option was (d) 'I never use this'. Using these questions, we selected participants with a stable alcohol consumption to perform a sensitivity analysis.

The FFQ has been validated against a 9-day diet record, and the Spearman correlation coefficient between the alcohol intake assessed by the questionnaire and that estimated by the diet record was 0.89 for all subjects and 0.85 for users of alcoholic beverages.²⁰ The reproducibility of the FFQ was established and the test-retest correlation was 0.90 for alcohol intake, and this correlation declined only 0.01-0.02 per year.²¹ This indicates that the single FFQ measurement was able to rank subjects according to alcohol intake and this ability dropped only slightly over time. The single FFQ measurement that is used in our cohort study can characterize dietary habits for a period of at least 5 years.²¹

Questionnaire data were key-entered and processed in a standardized manner, blinded with respect to case/subcohort status in order to minimize observer bias in coding and data interpretation.

Figure 2.1 Flow diagram of subcohort members and Barrett's esophagus cases on whom the analyses were based. PALGA, nationwide network and registry of histo- and cytopathology in the Netherlands; SIM, specialized intestinal metaplasia.

Statistical analysis

To evaluate the potential influence of prediagnostic BE at baseline on smoking and alcohol consumption habits and BMI, BE cases were categorized according to the year of follow-up in which they were diagnosed. A t-test was used to compare the differences in mean levels of these exposures between early (0-2 y) and late (2-16.3 y) follow-up. For the t-test, the exposures were ln (natural logarithm) transformed to normalize the distributions. The average daily alcohol consumption among early cases (2.8 g ethanol) was statistically significantly lower when compared with late cases (10.2 g ethanol, figures for cases with SIM). Therefore, we decided to exclude these early cases from all analyses, to prevent bias. Additionally, subcohort members and cases with inconsistent or incomplete dietary questionnaire data²⁰ and those with missing data on the confounders were excluded. Complete data were available for 3866 subcohort members, 370 BE cases fulfilling the primary case definition, and 500 BE cases fulfilling the secondary case definition (Figure 2.1).

The multivariable regression models included the following variables: age, sex, BMI (kg/m^2), cigarette smoking (current, frequency and duration), and alcohol consumption (g/day). Exact model specifications can be found in the table footnotes. The following variables were potential confounders, but were not included in the models because they did not change the incidence rate ratio (RR) by >5%: highest level of education, family history of esophageal or gastric cancer, reported long-term (>0.5 y) use of nonsteroidal anti-inflammatory drugs (NSAIDs) or aspirin, or lower esophageal sphincter relaxing medication,^{22,23} non-occupational physical activity, daily intakes of vegetables and fruit.

Multivariable adjusted incidence rate ratios (RR) and corresponding 95% confidence intervals (CI) were estimated using Cox proportional hazards models.²⁴ The Stata 9.2 statistical software package (StataCorp, College Station, Texas, USA) was used for analysis. Standard errors were estimated using the robust Huber-White sandwich estimator to account for additional variance introduced by sampling from the cohort. This method is equivalent to the variance-covariance estimator by Barlow.²⁵ We tested the proportional hazards assumption using the scaled Schoenfeld residuals.²⁶ Tests for dose-response trends were assessed by fitting ordinal exposure variables as continuous terms. Two-sided p values are reported throughout the article. Interaction with sex was assessed by including a cross-product term in the model. If the interaction was statistically significant, we only presented results stratified by sex.

RESULTS

Characteristics

With respect to the characteristics mentioned in Table 2.1, there were no important differences between the BE cases fulfilling the primary and secondary case definitions. The most marked differences between subcohort members and cases are the following. Cases were more likely to be men and former smokers than subcohort members, while cases had a slightly lower ethanol intake. The BMI at baseline and the change in BMI from age 20 were somewhat higher among cases than among the subcohort. Furthermore, cases were somewhat less physically active and consumed somewhat less fruit and vegetables than the subcohort. NSAIDs and aspirin, and lower esophageal sphincter relaxing medication were more likely used by cases than subcohort members.

Cox regression results

All results from Cox regression analyses were very similar for BE cases defined by primary and secondary case definitions. We therefore only showed the results based on the BE cases that met the primary case definition. Likewise, only multivariable

adjusted results are shown, as these were very similar to the age-adjusted results (see Supplemental Tables 2.1, 2.2, 2.3).

Table 2.1 Characteristics of cases and subcohort members in the Netherlands Cohort Study (1986–2002).

Characteristic	Subcohort (n=3866) ^a	Barrett's esophagus cases	
		SIM (n=370) ^a	SIM or unknown metaplasia (n=500) ^a
	Mean (SD) ^b	Mean (SD) ^b	Mean (SD) ^b
Age at baseline (years)	61.3 (4.2)	61.1 (4.3)	61.3 (4.2)
Men (%)	49.0	57.3	56.2
Cigarette smoking status			
Never smoker (%)	37	33	33
Former smoker (%)	36	46	44
Current smoker (%)	37	21	23
Ever cigarette smokers:			
Frequency of cigarette smoking (n/day)	15.3 (10.3)	16.0 (10.8)	16.1 (10.4)
Duration of cigarette smoking (years)	31.5 (12.2)	30.8 (11.8)	30.9 (12.0)
Pack-years of cigarette smoking (n)	22.6 (17.8)	22.6 (16.7)	22.9 (16.8)
Abstainer from alcohol (%)	23	20	21
Alcohol consumers:			
Ethanol intake (g/day)	13.5 (15.0)	12.9 (15.0)	13.1 (14.9)
Beer intake (glasses/day)	0.3 (0.8)	0.3 (0.7)	0.3 (0.7)
Wine intake (glasses/day)	0.5 (0.8)	0.5 (0.7)	0.5 (0.8)
Liquor intake (glasses/day)	0.5 (0.8)	0.5 (0.8)	0.5 (0.8)
BMI at baseline (kg/m ²)	25.1 (3.0)	25.4 (2.8)	25.5 (2.8)
BMI at age 20 years (kg/m ²)	21.5 (2.6)	21.6 (2.5)	21.6 (2.6)
BMI change from age 20 to baseline (kg/m ²)	3.6 (3.3)	3.8 (3.0)	3.8 (3.1)
Non-occupational physical activity (min/day)	74 (61)	69 (53)	70 (55)
Fruit consumption (g/day)	178 (120)	171 (118)	172 (116)
Vegetable consumption (g/day)	195 (82)	189 (77)	184 (76)
Highest level of education			
Primary (%)	28	28	31
Lower vocational (%)	22	20	20
Secondary and medium vocational (%)	36	36	34
University and higher vocational (%)	14	16	15
Family history of esophageal or gastric cancer (%)	7	7	7
Use of NSAIDs and aspirin (%) ^c	7	10	10
Use of LES relaxing medication (%) ^c	14	17	18

^a Presented are the number of subcohort members or cases with complete data on age, sex, cigarette smoking (current yes/no, number of cigarettes smoked daily, number of smoking years), alcohol consumption and body mass index. Subcohort members and cases with incomplete or inconsistent questionnaire data are excluded, as well as the first two years of follow-up; ^b For categorical variables a percentage is presented; ^c Self reported use during more than 0.5 year; BMI, body mass index; IQR, interquartile range; LES, lower esophageal sphincter; NSAIDs, non-steroidal anti-inflammatory drugs; SD, standard deviation; SIM, specialized intestinal metaplasia.

Overweight

Women who were overweight (BMI: 25-<30) or obese (BMI: ≥ 30) at baseline were at increased risk of BE. Compared with normal weight (BMI 18.5-<25) women, the RRs were 1.73 (95% CI 1.22-2.44) for overweight and 1.67 (95% CI 0.96-2.90) for obese women. The results for men were significantly different from those for women (p interaction=0.01). Men who were overweight or obese at baseline were not at increased risk of BE: RRs were 0.85 (95% CI 0.63-1.14) and 1.21 (95% CI 0.60-2.47), respectively (Table 2.2).

An interaction with sex was also found for BMI at age 20 (p interaction=0.01). A positive association was found for women (RR for BMI ≥ 25 at age 20 $\gamma=1.56$, 95% CI 0.79-3.10), p trend=0.05], while an inverse association was found for men (RR for BMI ≥ 25 at age 20 $\gamma=0.37$, 95% CI 0.15-0.87, p trend=0.02). A change in BMI during adulthood was not associated with risk of BE in men, but a positive trend was observed in women (p trend=0.01), mainly because women who lost weight during adulthood were at decreased risk (RR=0.33, 95% CI 0.15-0.75) when compared with those who gained 0-<4 BMI points.

When RRs for BMI at baseline were additionally adjusted for pant/skirt size, the associations for women were somewhat attenuated, and for men the associations were similar when compared with analyses without adjustment for pant/skirt size. Analyses with pant/skirt size as independent variable showed a positive association in women (p trend=0.01), which disappeared after adjustment for BMI at baseline. For men, no association was observed for pant/skirt size (p trend=0.73), and adjustment for BMI at baseline did not change this observation (Table 2.2).

Cigarette smoking

In the multivariable regression analyses on cigarette smoking (Table 2.3), we did not observe any statistically significant differences between associations for men and women, which permitted us to report the results for both sexes combined. Nearly all RRs associated with aspects of cigarette smoking were above unity, although these were mostly not statistically significant. Former smokers were at highest risk of BE (RR=1.33, 95% CI 1.00-1.77) when compared with never smokers, but current smokers were not at higher risk. Smoking frequency was found to be related to a non-significantly higher risk of BE (p trend=0.46). A longer duration of cigarette smoking was associated with an increasing risk of BE (p trend=0.03). Results on the association between pack-years of smoking and BE showed increased RRs, but only for subjects who had smoked 20-<40 pack-years the RR was statistically significant (RR=1.50, 95% CI 1.04-2.17, p trend=0.08). The results of analyses on smoking cessation did not indicate a lower risk of BE with increasing duration of smoking cessation (p trend=0.82).

Table 2.2 Association (multivariable-adjusted^a) between overweight and risk of Barrett's esophagus with SIM; Netherlands Cohort Study (1986-2002).

	categorical median	Men			Women			p inter-action ^b
		person time at risk in subcohort (y)	no. of cases	RR (95% CI)	person time at risk in subcohort (y)	no. of cases	RR (95% CI)	
BMI at baseline (kg/m ²)								
18.5- <25	23.3	12267	119	1 (reference)	14327	65	1 (reference)	
25- <30	26.6	9801	83	0.85 (0.63-1.14)	9151	75	1.72 (1.22-2.44)	
≥ 30	31.5	808	10	1.21 (0.60-2.47) ^c	2288	18	1.67 (0.96-2.90)	0.01
continuous, 1 kg/m ² increments		22876	212	p trend ^d 0.73 (0.93-1.05) ^c	25766	158	p trend 0.01 (1.03-1.11)	0.03
BMI at age 20 years (kg/m ²)								
<20	18.7	3603	32	0.72 (0.45-1.13)	7057	36	1.06 (0.62-1.80)	
20-<21.5	20.8	5027	64	1 (reference)	5190	25	1 (reference)	
21.5-<23	22.2	4885	37	0.61 (0.40-0.94)	5386	38	1.45 (0.86-2.46)	
23-<25	23.8	3578	25	0.56 (0.34-0.91)	4076	31	1.59 (0.92-2.76)	
≥25	26.0	1323	6	0.37 (0.15-0.87) ^c	1775	14	1.56 (0.79-3.10) ^c	0.01
continuous, 1 kg/m ² increments		18417	164	p trend 0.02 (0.87-0.99)	23485	144	p trend 0.05 (1.01-1.13)	0.001
Change in BMI after 20 years of age (kg/m ²) ^e								
<0	-1.3	1763	8	0.56 (0.26-1.20)	2662	8	0.33 (0.15-0.75)	
0-<4	2.2	9908	95	1 (reference)	10499	67	1 (reference)	
4-<8	5.5	5799	51	0.77 (0.52-1.15)	7675	51	1.12 (0.76-1.64)	
≥8	9.4	947	10	0.83 (0.39-1.78)	2648	18	1.21 (0.69-2.10)	0.95
continuous, 1 kg/m ² increments		18417	164	p trend 0.79 (0.92-1.06)	23485	144	p trend 0.01 (1.02-1.10)	0.92
BMI at baseline (kg/m ²), additionally adjusted for pant/skirt size ^f								
18.5- <25	23.3	11349	113	1 (reference)	14228	65	1 (reference)	
25- <30	26.6	8925	77	0.81 (0.57-1.15)	9037	75	1.56 (1.02-2.39)	
≥ 30	31.5	661	10	1.35 (0.59-3.06) ^c	2205	18	1.38 (0.65-2.89)	0.01
continuous, 1 kg/m ² increments		20935	200	p trend 0.78 (0.92-1.08) ^c	25469	158	p trend 0.23 (0.99-1.13)	0.07

	Men				Women			
	categorical median	person time at risk in subcohort (y)	no. of cases	RR (95% CI)	person time at risk in subcohort (y)	no. of cases	RR (95% CI)	p interaction ^b
Pant/skirt size as a proxy for waist circumference (men/women)^f								
≤48 / ≤40		3085	30	1.20 (0.73-1.98)	4636	12	0.44 (0.22-0.85)	
50-51 / 42		4834	40	1 (reference)	6487	39	1 (reference)	
52-53 / 44		7188	73	1.22 (0.81-1.84)	7049	55	1.25 (0.82-1.92)	
54-55 / 46-48		3961	37	1.11 (0.69-1.78) ^c	6487	46	1.10 (0.70-1.71)	
≥56 / ≥50		1867	20	1.28 (0.72-2.27) ^c	810	6	1.23 (0.50-3.04)	
				p trend 0.73			p trend 0.01	
continuous		20935	200	1.02 (0.91-1.15)	25469	158	1.16 (1.05-1.28)	
Pant/skirt size as a proxy for waist circumference (men/women), additionally adjusted for BMI at baseline^f								
≤48 / ≤40		3085	30	1.19 (0.72-1.98) ^c	4636	12	0.51 (0.26-1.01)	
50-51 / 42		4834	40	1 (reference)	6487	39	1 (reference)	
52-53 / 44		7188	73	1.23 (0.81-1.87)	7049	55	1.08 (0.70-1.69)	
54-55 / 46-48		3961	37	1.12 (0.68-1.85)	6487	46	0.76 (0.44-1.33)	
≥56 / ≥50		1867	20	1.26 (0.67-2.38)	810	6	0.52 (0.15-1.83)	
				p trend 0.76			p trend 0.66	
continuous		20935	200	1.03 (0.89-1.18)	25469	158	1.03 (0.87-1.23)	

^a adjusted for age (years), cigarette smoking (current smoking status (yes/no), frequency (number of cigarettes/day), and duration (years)), alcohol consumption (g/day);

^b p-value for interaction between sex and measure of overweight (BMI, BMI at age 20, BMI change or pant/skirt size), based on cross product term in the Cox proportional hazard model; ^c proportional hazards assumption was violated for this analysis; ^d tests for dose-response trends were assessed by fitting ordinal variables as continuous terms in the Cox proportional hazard model; ^e additionally adjusted for BMI at age 20 years; ^f pant size (men) corresponds to the following standard waist circumferences: ≤50 = ≤88 cm; 50-51 = 88 cm; 52-53 = 93 cm; 54-55 = 98 cm; ≥56 = ≥103 cm; skirt size (women) corresponds to the following standard waist circumferences: ≤40 = ≤74 cm; 40 = 74 cm; 42 = 78 cm; 44 = 82 cm; 46 = 86 cm; 48 = 91 cm; ≥50 = ≥96 cm. BMI, body mass index; CI, confidence interval; RR, incidence rate ratio; SIM specialized intestinal metaplasia.

Table 2.3 Association (multivariable-adjusted^b) between cigarette smoking and risk of Barrett's esophagus with SIM; Netherlands Cohort Study (1986-2002).

	Total				Men			Women			p inter-action ^b
	category	person time at risk in subcohort cases (y)	no. of cases	RR (95% CI)	person time at risk in subcohort cases (y)	no. of cases	RR (95% CI)	person time at risk in subcohort cases (y)	no. of cases	RR (95% CI)	
Smoking status ^a											
never smokers	0	18888	121	1 (reference)	3380	27	1 (reference)	15508	94	1 (reference)	
former smokers		17390	170	1.33 (1.00-1.77)	12120	127	1.35 (0.87-2.10)	5270	43	1.55 (1.03-2.33)	
current smokers		12365	79	0.93 (0.68-1.28)	7376	58	1.04 (0.64-1.69)	4989	21	0.81 (0.49-1.36)	0.45
				p trend ^c 0.61			p trend 0.71			p trend 0.93	
Frequency of cigarette smoking (no./day) ^d											
no cigarette smoker	0	18888	121	1 (reference)	3380	27	1 (reference)	15508	94	1 (reference)	
>0 - <20	10	18819	149	1.16 (0.75-1.81)	10965	101	0.97 (0.50-1.86)	7853	48	1.69 (0.91-3.13)	
≥20	20	10936	100	1.24 (0.74-2.08)	8531	84	1.02 (0.50-2.10)	2405	16	1.89 (0.78-4.59)	0.99
				p trend 0.46			p trend 0.79			p trend 0.17	
continuous, 10 cigarettes/day increments	48642	48642	370	1.05 (0.93-1.19)	22876	212	1.05 (0.92-1.20)	25766	158	1.09 (0.80-1.50)	0.83
Duration of cigarette smoking (years) ^e											
no cigarette smoker	0	18888	121	1 (reference)	3380	27	1 (reference)	15508	94	1 (reference)	
>0 - <20	12	5724	41	1.05 (0.68-1.60)	2987	20	0.79 (0.41-1.52)	2737	21	1.55 (0.88-2.73)	
20 - <40	30	14353	129	1.37 (0.96-1.94)	9095	95	1.35 (0.81-2.25)	5258	34	1.56 (0.81-3.03)	
≥40	43	9677	79	1.51 (0.97-2.34)	7413	70	1.63 (0.90-2.95)	2263	9	1.13 (0.42-3.04)	0.20
				p trend 0.03			p trend 0.03			p trend 0.41	
continuous, 10 years increments	48642	48642	370	1.07 (0.97-1.19)	22876	212	1.10 (0.96-1.25)	25766	158	1.08 (0.87-1.33)	0.43
Pack-years of cigarette smoking ^f											
no cigarette smoker	0	18888	121	1 (reference)	3380	27	1 (reference)	15508	94	1 (reference)	
>0 - <20	9	15872	125	1.27 (0.94-1.72)	8787	79	1.24 (0.78-1.97)	7085	46	1.55 (1.01-2.38)	
20 - <40	28	9612	87	1.50 (1.04-2.17)	7220	71	1.51 (0.92-2.47)	2393	16	1.80 (0.94-3.45)	
≥40	48	4270	37	1.44 (0.91-2.28)	3490	35	1.59 (0.89-2.84)	781	2	0.65 (0.15-2.90)	0.55
				p trend 0.08			p trend 0.09			p trend 0.37	
continuous, 10 pack-years increments	48642	48642	370	1.05 (0.98-1.12)	22876	212	1.05 (0.98-1.13)	25766	158	1.08 (0.91-1.28)	0.15

	Total			Men			Women		
	categorical median	person timeno. at risk in subcohort (y)	RR (95% CI)	person time no. at risk in subcohort (y)	RR (95% CI)	person timeno. at risk in subcohort (y)	RR (95% CI)	person timeno. at risk in subcohort (y)	p inter-action ^b
Smoking cessation^a									
never smokers		1888	121 1	(reference)		3380	27 1	(reference)	
stopped ≥ 20 years ago	25	5413	47 1.16	(0.79-1.71)		3972	37 1.18	(0.70-1.99)	
stopped 10 - <20 years ago	14	5960	64 1.46	(1.03-2.07)		4205	53 1.63	(0.99-2.67)	
stopped >0 - <10 years ago	5	5985	58 1.32	(0.91-1.91)		3943	37 1.23	(0.72-2.10)	
current smokers	0	12365	79 0.93	(0.68-1.28)	p trend 0.82	7376	58 1.04	(0.64-1.69)	p trend 0.78
									p trend 0.79

^a all analyses were adjusted for: age (years), alcohol consumption (g/day), body mass index (kg/m²); ^b p-value for interaction between sex and cigarette smoking, based on cross product term in the Cox proportional hazard model; ^c tests for dose-response trends were assessed by fitting ordinal variables as continuous terms in the Cox proportional hazard model; ^d additionally adjusted for current smoking status (yes/no) and smoking duration (years); ^e additionally adjusted for current smoking status (yes/no) and smoking frequency (number of cigarettes/day); ^f additionally adjusted for current smoking status (yes/no). CI, confidence interval; RR, incidence rate ratio; SIM specialized intestinal metaplasia.

Table 2.4 Association (multivariable-adjusted^a) between alcohol consumption and risk of Barrett's esophagus with SIM; Netherlands Cohort Study (1986-2002).

	Total			Men			Women			p inter- action ^b			
	category	person time at risk in subcohort (y)	no. of cases	RR	(95% CI)	person time at risk in subcohort (y)	no. of cases	RR	(95% CI)				
Alcohol consumption (g ethanol/day)													
abstainer	0	11282	75	1	(reference)	3243	24	1	(reference)	8039	51	1	(reference)
>0<5	2	14416	122	1.22	(0.90-1.65)	4860	50	1.37	(0.81-2.31)	9556	72	1.19	(0.82-1.73)
5<15	9	10925	78	0.94	(0.67-1.31) ^c	6026	58	1.28	(0.77-2.13) ^c	4899	20	0.66	(0.38-1.16)
15<30	22	7692	64	1.00	(0.69-1.45)	5335	52	1.26	(0.75-2.13)	3273	15	0.75	(0.40-1.41) ^d
>=30	40	4327	31	0.82	(0.51-1.29)	3411	28	1.02	(0.57-1.84)				0.35
					p trend ^e 0.16				p trend 0.54				
continuous, 10 grams ethanol/day increments		48642	370	0.95	(0.87-1.03)	22876	212	0.97	(0.891.07)	25766	158	0.84	p trend 0.12 (0.65-1.08)
Alcohol consumption (g ethanol/day)													
Stable users ^f													
abstainer	0	9099	58	1	(reference)	2513	17	1	(reference)	6586	41	1	(reference)
>0<5	2	8447	80	1.43	(1.00-2.06) ^c	3133	34	1.60	(0.85-3.00) ^c	5313	46	1.43	(0.92-2.23)
5<15	9	6694	46	0.96	(0.64-1.45) ^c	3904	35	1.36	(0.73-2.53) ^c	2790	11	0.62	(0.30-1.27)
15<30	22	4239	38	1.19	(0.76-1.88) ^c	3022	33	1.71	(0.90-3.26) ^c	1734	6	0.53	(0.21-1.35) ^d
>=30	40	2346	17	0.90	(0.50-1.62)	1829	16	1.29	(0.62-2.67)				0.27
					p trend 0.42				p trend 0.73				
continuous, 10 g ethanol/day increments		30825	239	0.97	(0.87-1.09)	14402	135	1.03	(0.91-1.15)	16423	104	0.73	p trend 0.05 (0.51-1.05)
Alcoholic beverages (glasses/day) ^g													
Beer													
no beer	0	32777	221	1	(reference)	9591	72	1	(reference)				
>0-1	0.2	12552	130	1.31	(1.01-1.69)	10162	121	1.60	(1.16-2.21)				
>1-2	1.4	2167	14	0.81	(0.45-1.48)	2043	14	0.95	(0.51-1.77)				
>2	3.7	1146	5	0.59	(0.23-1.51)	1080	5	0.67	(0.26-1.75)				
					p trend 0.10				p trend 0.10				
continuous, 1 glass/day increments		48642	370	0.86	(0.69-1.08)	22876	212	0.86	(0.69-1.07)				

	Total				Men				Women				p inter- action ^b
	categorical median	person time at risk in subcohort (y)	no. of cases	RR (95% CI)	person time at risk in subcohort (y)	no. of cases	RR (95% CI)	person time at risk in subcohort (y)	no. of cases	RR (95% CI)			
Wine													
no wine	0	22196	161	1 (reference)	11012	89	1 (reference)	11184	72	1 (reference)			
>0-1	0.2	20214	166	1.19 (0.94-1.50)	9050	97	1.34 (0.98-1.82)	11163	69	1.11 (0.77-1.62)			
>1-2	1.4	4097	30	1.15 (0.73-1.79) ^c	1800	17	1.23 (0.68-2.24) ^c	3418	17	1.48 (0.72-3.01) ^d		0.50	
>2	2.7	2135	13	1.03 (0.53-2.01)	1014	9	1.20 (0.54-2.69)						
				p trend 0.91			p trend 0.72				p trend 0.29		
continuous, 1 glass/day increments		48642	370	1.04 (0.86-1.28) ^c	22876	212	1.06 (0.84-1.33) ^c	25766	158	1.71 (0.83-3.55)		0.42	
Liquor													
no liquor	0	24989	169	1 (reference)	8300	70	1 (reference)	16689	99	1 (reference)			
>0-1	0.2	17954	149	1.16 (0.91-1.48) ^c	9790	92	1.15 (0.82-1.62) ^c	8164	57	1.20 (0.84-1.71)			
>1-2	1.9	4068	37	1.35 (0.86-2.11)	3316	36	1.48 (0.89-2.47)	913	2	0.53 (0.13-2.14) ^d		0.44	
>2	2.8	1631	15	1.60 (0.79-3.24)	1470	14	1.50 (0.68-3.28)						
				p trend 0.17			p trend 0.17				p trend 0.51		
continuous, 1 glass/day increments		48642	370	1.10 (0.89-1.36) ^c	22876	212	1.13 (0.89-1.42)	25766	158	0.70 (0.35-1.41)		0.14	

^a Adjusted for age (years), cigarette smoking (current smoking status (yes/no), frequency (number of cigarettes/day), and duration (years)), body mass index (kg/m²); ^b p-value for interaction between sex and alcohol consumption, based on cross product term in the Cox proportional hazard model; ^c Proportional hazards assumption was violated for this analysis; ^d For analyses on women, the highest two categories of consumption were combined, because of low case numbers; ^e Tests for dose-response trends were assessed by fitting ordinal variables as continuous terms in the Cox proportional hazard model; ^f Subjects who had not changed their alcohol consumption habits in the 5 years before baseline; ^g Additionally adjusted for ethanol intake; ^h It was not possible to perform analyses on beer consumption in women, because too few women consumed beer. CI, confidence interval; RR, incidence rate ratio; SIM, specialized intestinal metaplasia.

Alcohol consumption

As can be seen in Table 2.4, there was no interaction between sex and alcohol consumption in the analyses of BE risk. Analyses of the association between alcohol intake and risk of BE indicated no increased risk. Subjects in the highest category of intake (≥ 30 g ethanol/day) had an RR of 0.82 (95% CI 0.51-1.29) when compared with abstainers. A sensitivity analysis was performed based on subjects who had a stable alcohol consumption in the period starting 5 years before baseline until baseline. This analysis included approximately 65% of the study population. Broadly speaking, the results were comparable with those based on the total study population.

Also shown in Table 2.4 are RRs associated with consumption of alcoholic beverages, adjusted for ethanol intake. The RRs for beer and wine consumption do not show clear inverse or positive associations. Increasing liquor consumption was associated with a non-significantly increased risk of BE (RR for >2 glasses/day=1.60, 95% CI 0.79-3.24, p trend=0.17).

DISCUSSION

In this large prospective cohort study, overweight was found to be a risk factor for BE in women, but not in men. Several aspects of cigarette smoking were associated with an increase in risk of BE, while alcohol consumption was not associated with risk of BE.

Overweight

Our observation of a positive association between increased BMI and risk of BE in women, is comparable with a recent observation in another prospective cohort study among women. They found obese women to be at increased risk of BE (OR 1.52, 95% CI 1.02-2.28). However, no association with overweight was found in that study (OR 0.92, 95% CI 0.66-1.27), while we did find a positive association with overweight. Three case-control studies showed an increased risk of BE for overweight and obese persons.⁷⁻⁹ These studies were based on predominantly male populations (60-100% male cases), thus it is likely that overweight and obese men were at increased risk, even though no separate results by sex were presented. We did not observe an increased risk in men. Previously, a positive association between overweight and risk of esophageal adenocarcinoma has also been described, in the NLCS (in women only)²⁷ and a meta-analysis.²⁸

Several possible biological mechanisms may be involved in the association between overweight and development of BE. Some studies observed that adjustment for gastroesophageal reflux disease caused attenuation or disappearance of the effect of increased BMI on BE risk,^{6,29} whereas in other studies this adjustment did not affect the RRs.^{5,10} Therefore, it is probable that the BMI acts through other mechanisms besides gastroesophageal reflux disease, as was also suggested recently in a

comprehensive review.³⁰ Another possible mechanism relates to the distribution of body fat. Abdominal fat is more metabolically active than subcutaneous fat, and secretes several factors that may be involved in systemic inflammation and cancer development.^{30,31} Two case-control studies observed positive associations between measures of abdominal overweight and BE risk.^{8,32} In our study, we did not observe an increased risk of BE for women with abdominal overweight, when total overweight was accounted for. However, the use of clothing size measures as a proxy for waist circumference may not have been optimal. Results from the Nurses' Health Study did not show an association for abdominal overweight either.¹⁰ Thus, there are some inconsistencies between observations on the role of abdominal overweight in the development BE.

Cigarette smoking

Our observation that several aspects of cigarette smoking were associated with an increased risk of BE are in agreement with results from some previous case-control studies.^{6,8} In an earlier analysis within the NLCS, we investigated the association of cigarette smoking with risk of esophageal adenocarcinoma.³³ The association of cigarette smoking with esophageal adenocarcinoma was stronger (RR for ≥ 40 pack-years=2.93 (95% CI 1.59-5.40) than the association with its precursor BE. This difference in strength of the associations with BE and esophageal adenocarcinoma is consistent with the literature.^{e.g. 5,8,12,34,35} This observation may indicate that smoking plays a role in the later stages of esophageal carcinogenesis: in the progression of BE to esophageal adenocarcinoma. In a population of BE patients, risk of progression was indeed found to be increased for patients who had smoked, although this was not statistically significant (RR=1.53 (95% CI 0.68-6.44)).³⁶ Further investigation into this topic may yield interesting insights.

Alcohol consumption

Our finding that there was no association between alcohol intake and risk of BE is consistent with results from most studies.^{8,11,13} Also, the findings are analogous to the null association between alcohol and risk of esophageal adenocarcinoma.^{33,34,37}

Wine consumption has been reported to be inversely associated with BE risk.^{11,13} However, within the NLCS, we could not confirm this association. The fact that we adjusted for total ethanol intake while the other two studies adjusted for beer and liquor intake, cannot explain the different findings, as there were no associations with ethanol, beer or liquor.

Strengths and limitations

An important strength of this study is its prospective character. The advantage of the prospective design is the relative insensitivity for selection and information bias

compared with a case-control design. Second, this study is one of the largest with respect to the number of BE cases, specifically the number of female cases. This large number of cases allowed the investigation of interaction and separate analyses for men and women. A third strength is the availability of extensive information about the exposure variables investigated, which allowed a detailed look at these exposures.

There are also some limitations to our study. The first is the lack of information on the presence of gastroesophageal reflux disease and the use of medications for this disease. Consequently, we were not able to investigate possible confounding effects of these factors or their possible intermediate role in the associations investigated. Second, the use of clothing size as a proxy measure for waist circumference is sub-optimal compared with measurement of waist circumference. Third, subcohort members who were not diagnosed with BE were assumed to be disease-free, but we were unable to verify this assumption. Therefore, there may be some BE cases missing in our dataset, which may have reduced the power. Finally, the power of the study also may have been reduced due to the incomplete coverage of the NLCS population by PALGA before 1991. Due to this incompleteness we may have missed some BE cases or the registered incidence date may be later than the true incidence date. We estimated that we may have missed 3% of the BE cases in our cohort at most.

Recommendations for further research

Most studies that investigated overweight, smoking, and alcohol consumption in relation to the risk of BE, had a case-control design. Reversed causation can be a problem in studies investigating etiology, specifically in studies with a case-control design. This is illustrated by our finding that cases diagnosed during the first 2 years of follow-up reported different alcohol consumption habits at baseline than later cases. Only one prospective cohort study previously reported on overweight and risk of BE, while we are the first prospective cohort study to report on alcohol and smoking. We therefore suggest that additional cohort studies investigate these factors in association with BE risk. Future studies should, whenever possible, investigate whether associations differ by sex. Differences in associations between sexes may help to explain the higher male-to-female ratio in esophageal adenocarcinoma compared with BE. As mentioned before, studying risk factors for progression of BE to esophageal adenocarcinoma also deserves further attention.

Supplemental Table 2.1 Association (age-adjusted) between overweight and risk of Barrett's esophagus with SIM; Netherlands Cohort Study (1986-2002).

	Men				Women			
	categorical median	person time at risk in subcohort (y)	no. of cases	RR (95% CI)	person time at risk in subcohort (y)	no. of cases	RR (95% CI)	p inter-action ^a
BMI at baseline (kg/m²)								
18.5- <25	23.3	12267	119	1 (reference)	14327	65	1 (reference)	
25- <30	26.6	9801	83	0.88 (0.65-1.18)	9151	75	1.79 (1.27-2.53)	
≥ 30	31.5	808	10	1.29 (0.64-2.60) ^b	2288	18	1.74 (1.01-3.01)	0.01
Continuous, 1 kg/m ² increments		22876	212	p trend ^c 0.90 1.00 (0.94-1.06) ^b	25766	158	p trend 0.002 1.07 (1.04-1.11)	0.04
BMI at age 20 years (kg/m²)								
<20	18.7	3603	32	0.71 (0.45-1.11)	7057	36	1.06 (0.63-1.80)	
20-<21.5	20.8	5027	64	1 (reference)	5190	25	1 (reference)	
21.5-<23	22.2	4885	37	0.60 (0.39-0.93)	5386	38	1.46 (0.87-2.47)	
23-<25	23.8	3578	25	0.56 (0.34-0.91)	4076	31	1.59 (0.92-2.75)	
≥25	26.0	1323	6	0.37 (0.16-0.88)	1775	14	1.64 (0.83-3.24)	0.01
continuous, 1 kg/m ² increments		18417	164	p trend 0.03 0.93 (0.87-0.99)	23485	144	p trend 0.04 1.07 (1.01-1.13)	0.001
Change in BMI after 20 years of age (kg/m²)^d								
<0	-1.3	1763	8	0.48 (0.23-1.00)	2662	8	0.47 (0.22-1.00)	
0-<4	2.2	9908	95	1 (reference)	10499	67	1 (reference)	
4-<8	5.5	5799	51	0.90 (0.63-1.29)	7675	51	1.04 (0.71-1.52)	
≥8	9.4	947	10	1.10 (0.55-2.20)	2648	18	1.07 (0.62-1.84)	0.96
continuous, 1 kg/m ² increments		18417	164	p trend 0.29 1.03 (0.98-1.08)	23485	144	p trend 0.11 1.03 (0.99-1.08)	0.97
BMI at baseline (kg/m²), additionally adjusted for pant/skirt size^e								
18.5- <25	23.3	11349	113	1 (reference)	14228	65	1 (reference)	
25- <30	26.6	8925	77	0.83 (0.59-1.17)	9037	75	1.61 (1.05-2.46)	
≥ 30	31.5	661	10	1.40 (0.62-3.16) ^b	2205	18	1.40 (0.67-2.95)	0.01
continuous, 1 kg/m ² increments		20935	200	p trend 0.87 1.00 (0.93-1.08) ^b	25469	158	p trend 0.20 1.06 (1.00-1.14)	0.09

	Men				Women			
	categorical median	person time at risk in subcohort (y)	no. of cases	RR (95% CI)	person time at risk in subcohort (y)	no. of cases	RR (95% CI)	p inter-action ^a
Pant/skirt size as a proxy for waist circumference (men/women)^e								
≤48 / ≤40		3085	30	1.18 (0.72-1.95)	4636	12	0.43 (0.22-0.83)	
50-51 / 42		4834	40	1 (reference)	6487	39	1 (reference)	
52-53 / 44		7188	73	1.23 (0.82-1.86)	7049	55	1.29 (0.84-1.97)	
54-55 / 46-48		3961	37	1.13 (0.71-1.81) ^b	6487	46	1.16 (0.74-1.81)	
≥56 / ≥50		1867	20	1.31 (0.74-2.33) ^b	810	6	1.23 (0.50-3.03)	
				p trend 0.61			p trend 0.002	
continuous		20935	200	1.03 (0.92-1.16)	25469	158	1.18 (1.07-1.29)	
Pant/skirt size as a proxy for waist circumference (men/women), additionally adjusted for BMI at baseline^e								
≤48 / ≤40		3085	30	1.17 (0.71-1.95) ^b	4636	12	0.51 (0.26-1.01)	
50-51 / 42		4834	40	1 (reference)	6487	39	1 (reference)	
52-53 / 44		7188	73	1.23 (0.81-1.88)	7049	55	1.10 (0.71-1.71)	
54-55 / 46-48		3961	37	1.13 (0.69-1.86)	6487	46	0.78 (0.45-1.36)	
≥56 / ≥50		1867	20	1.28 (0.68-2.42)	810	6	0.49 (0.14-1.70)	
				p trend 0.70			p trend 0.59	
continuous		20935	200	1.03 (0.90-1.19)	25469	158	1.04 (0.87-1.23)	

^a p-value for interaction between sex and measure of overweight (BMI, BMI at age 20, BMI change or pant/skirt size), based on cross product term in the Cox proportional hazard model; ^b Proportional hazards assumption was violated for this analysis; ^c Tests for dose-response trends were assessed by fitting ordinal variables as continuous terms in the Cox proportional hazard model; ^d Additionally adjusted for BMI at age 20 years; ^e Pant size (men) corresponds to the following standard waist circumferences: ≤50 = ≤88 cm; 50-51 = 88 cm; 52-53 = 93 cm; 54-55 = 98 cm; ≥56 = ≥103 cm; skirt size (women) corresponds to the following standard waist circumferences: ≤40 = ≤74 cm; 40 = 74 cm; 42 = 78 cm; 44 = 82 cm; 46 = 86 cm; 48 = 91 cm; ≥50 = ≥96 cm. BMI, body mass index; CI, confidence interval; RR, incidence rate ratio; SIM specialized intestinal metaplasia.

Supplemental Table 2.2 Association (age-adjusted) between cigarette smoking and risk of Barrett's esophagus with SIM; Netherlands Cohort Study (1986-2002).

	Total			Men			Women			p inter-action ^a
	categorical median	person time no. of cases at risk in subcohort (y)	RR (95% CI)	person time no. of cases at risk in subcohort (y)	RR (95% CI)	person time no. of cases at risk in subcohort (y)	person time no. of cases at risk in subcohort (y)	RR (95% CI)	p trend	
Smoking status										
never smokers	18888	121	1 (reference)	3380	27	1 (reference)	15508	94	1 (reference)	
former smokers	17390	170	1.29 (0.97-1.70)	12120	127	1.33 (0.86-2.07)	5270	43	1.37 (0.93-2.01)	
current smokers	12365	79	0.89 (0.65-1.21) p trend ^b 0.40	7376	58	1.02 (0.63-1.65)	4989	21	0.71 (0.44-1.17)	0.47
									p trend 0.66	
Frequency of cigarette smoking (n/day)										
no cigarette smoker	18888	121	1 (reference)	3380	27	1 (reference)	15508	94	1 (reference)	
>0 - <20	18819	149	1.08 (0.82-1.42)	10965	101	1.17 (0.75-1.84)	7853	48	1.03 (0.71-1.49)	
≥20	10936	100	1.19 (0.87-1.63) p trend 0.29	8531	84	1.28 (0.81-2.02)	2405	16	1.14 (0.65-1.98)	0.90
									p trend 0.68	
continuous, 10 cigarettes/day increments	48642	370	1.07 (0.96-1.18)	22876	212	1.08 (0.96-1.21)	25766	158	1.02 (0.83-1.27)	0.67
Duration of cigarette smoking (years)										
no cigarette smoker	18888	121	1 (reference)	3380	27	1 (reference)	15508	94	1 (reference)	
>0 - <20	5724	41	0.99 (0.67-1.47)	2987	20	0.85 (0.46-1.57)	2737	21	1.29 (0.78-2.14)	
20 - <40	14353	129	1.21 (0.91-1.60)	9095	95	1.34 (0.85-2.10)	5258	34	1.10 (0.72-1.68)	
≥40	9677	79	1.05 (0.76-1.44) p trend 0.43	7413	70	1.22 (0.77-1.96)	2263	9	0.66 (0.33-1.34)	0.20
									p trend 0.16	
continuous, 10 years increments	48642	370	1.01 (0.94-1.07)	22876	212	1.03 (0.95-1.13)	25766	158	0.97 (0.88-1.08)	0.40

		Total			Men			Women			p inter- action ^a
	categorical median	person time at risk in subcohort (y)	no. of cases	RR (95% CI)	person time at risk in subcohort (y)	no. of cases	RR (95% CI)	person time at risk in subcohort (y)	no. of cases	RR (95% CI)	
Pack-years of cigarette smoking											
no cigarette smoker	0	18888	121	1 (reference)	3380	27	1 (reference)	15508	94	1 (reference)	
>0 - <20	9	15872	125	1.08 (0.82-1.43)	8787	79	1.14 (0.72-1.81)	7085	46	1.09 (0.75-1.59)	
20 - <40	28	9612	87	1.18 (0.85-1.63)	7220	71	1.27 (0.79-2.03)	2393	16	1.14 (0.66-1.99)	
≥40	48	4270	37	1.11 (0.74-1.67)	3490	35	1.31 (0.77-2.23)	781	2	0.43 (0.10-1.79)	0.56
				p trend 0.46			p trend 0.27			p trend 0.66	
continuous, 10 pack-years increments		48642	370	1.02 (0.96-1.08)	22876	212	1.03 (0.96-1.10)	25766	158	0.98 (0.85-1.12)	0.53
Smoking cessation											
never smokers		18888	121	1 (reference)	3380	27	1 (reference)	15508	94	1 (reference)	
stopped ≥ 20 years	25	5413	47	1.11 (0.76-1.63)	3972	37	1.17 (0.69-1.98)	1441	10	1.15 (0.58-2.29)	
stopped 10 - <20 years	14	5960	64	1.41 (1.00-1.98)	4205	53	1.61 (0.98-2.63)	1755	11	1.06 (0.55-2.04)	
stopped >0 - <10 years	5	5985	58	1.29 (0.90-1.86)	3943	37	1.20 (0.71-2.03)	2042	21	1.73 (1.04-2.89)	
current smokers	0	12365	79	0.88 (0.65-1.20)	7376	58	1.02 (0.63-1.65)	4989	21	0.72 (0.44-1.17)	0.34
				p trend 0.61			p trend 0.72			p trend 0.73	

^a p-value for interaction between sex and cigarette smoking, based on cross product term in the Cox proportional hazard model; ^b tests for dose-response trends were assessed by fitting ordinal variables as continuous terms in the Cox proportional hazard model. CI, confidence interval; RR, incidence rate ratio; SIM specialized intestinal metaplasia.

Supplemental Table 2.3 Association (age-adjusted) between alcohol consumption and risk of Barrett's esophagus with SIM, Netherlands Cohort Study (1986-2002).

	Total				Men				Women				p inter-action ^a
	category median	person time at risk in subcohort (y)	no. of cases	RR (95% CI)	person time at risk in subcohort (y)	no. of cases	RR (95% CI)	person time at risk in subcohort (y)	no. of cases	RR (95% CI)			
Alcohol consumption (g ethanol/day)													
abstainer	0	11282	75	1 (reference)	3243	24	1 (reference)	8039	51	1 (reference)			
>0-<5	2	14416	122	1.24 (0.92-1.68)	4860	50	1.40 (0.83-2.34)	9556	72	1.18 (0.81-1.72)			
5-<15	9	10925	78	0.96 (0.68-1.33) ^b	6026	58	1.32 (0.80-2.19) ^b	4899	20	0.64 (0.38-1.09)			
15-<30	22	7692	64	1.04 (0.73-1.49)	5335	52	1.33 (0.79-2.21) ^b	3273	15	0.73 (0.40-1.31) ^c	0.27		
>=30	40	4327	31	0.86 (0.55-1.34) p trend ^d 0.22	3411	28	1.11 (0.62-1.97)						
continuous, 10 grams ethanol/day increments		48642	370	0.96 (0.88-1.04)	22876	212	0.98 (0.90-1.07) p trend 0.75	25766	158	0.82 (0.65-1.04)	p trend 0.06	0.16	
Alcohol consumption (g ethanol/day), stable users ^e													
Abstainer	0	9099	58	1 (reference)	2513	17	1 (reference)	6586	41	1 (reference)			
>0-<5	2	8447	80	1.43 (1.00-2.04) ^b	3133	34	1.64 (0.89-3.03) ^b	5313	46	1.38 (0.89-2.15)			
5-<15	9	6694	46	0.96 (0.64-1.44) ^b	3904	35	1.39 (0.75-2.57) ^b	2790	11	0.63 (0.32-1.25)			
15-<30	22	4239	38	1.20 (0.78-1.86) ^b	3022	33	1.70 (0.91-3.17) ^b	1734	6	0.56 (0.23-1.35) ^c	0.21		
>=30	40	2346	17	0.94 (0.53-1.66) p trend 0.48	1829	16	1.34 (0.65-2.74)						
continuous, 10 g ethanol/day increments		30825	239	0.98 (0.88-1.09)	14402	135	1.03 (0.92-1.15) p trend 0.68	16423	104	0.74 (0.53-1.03)	p trend 0.04	0.07	
Alcoholic beverages (glasses/day) ^f													
Beer													
no beer	0	32777	221	1 (reference)	9591	72	1 (reference)						
>0-1	0.2	12552	130	1.33 (1.03-1.72)	10162	121	1.62 (1.17-2.23)					8	
>1-2	1.4	2167	14	0.84 (0.46-1.53)	2043	14	0.96 (0.51-1.80)						
>2	3.7	1146	5	0.60 (0.24-1.51) p trend 0.10	1080	5	0.67 (0.26-1.72)						
continuous, 1 glass/day increments		48642	370	0.87 (0.70-1.08)	22876	212	0.86 (0.69-1.07) p trend 0.09						

	Total			Men			Women			p inter-action ^a
	categorical median	person time at risk in subcohort (y)	no. of cases	RR	(95% CI)	person time at risk in subcohort (y)	no. of cases	RR	(95% CI)	
Wine										
no wine	0	22196	161	1	(reference)	11012	89	1	(reference)	
>0-1	0.2	20214	166	1.17	(0.93-1.48)	9050	97	1.33	(0.98-1.81)	
>1-2	1.4	4097	30	1.12	(0.72-1.76) ^b	1800	17	1.22	(0.68-2.22) ^b	0.46
>2	2.7	2135	13	0.99	(0.51-1.92)	1014	9	1.19	(0.54-2.65)	
					p trend 0.98				p trend 0.71	
continuous, 1 glass/day increments		48642	370	1.03	(0.84-1.25) ^b	22876	212	1.05	(0.84-1.32) ^b	0.37
									p trend 0.33	
									158	1.61 (0.81-3.22)
Liquor										
no liquor	0	24989	169	1	(reference)	8300	70	1	(reference)	
>0-1	0.2	17954	149	1.17	(0.92-1.49) ^b	9790	92	1.15	(0.82-1.61) ^b	
>1-2	1.9	4068	37	1.36	(0.87-2.12)	3316	36	1.48	(0.90-2.46)	0.42
>2	2.8	1631	15	1.65	(0.82-3.32)	1470	14	1.54	(0.70-3.36)	
					p trend 0.14				p trend 0.15	
continuous, 1 glass/day increments		48642	370	1.11	(0.90-1.36) ^b	22876	212	1.13	(0.90-1.43)	0.12
									p trend 0.54	
									158	0.72 (0.37-1.41)

^a p-value for interaction between sex and alcohol consumption based on cross product term in the Cox proportional hazard model; ^b Proportional hazards assumption was violated in this analysis; ^c For analyses on women, the highest two categories of consumption were combined, because of low case numbers; ^d Tests for dose-response trends were assessed by fitting ordinal variables as continuous terms in the Cox proportional hazard model; ^e Subjects who had not changed their alcohol consumption habits in the 5 years before baseline; ^f Additionally adjusted for ethanol intake; ^g It was not possible to perform analyses on beer consumption in women, because too few women consumed beer. CI, confidence interval; RR, incidence rate ratio; SIM specialized intestinal metaplasia.

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3

Toenail selenium status and the risk of Barrett's esophagus: the Netherlands Cohort Study

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ABSTRACT

Objective

To investigate the association between selenium and the risk of Barrett's esophagus (BE), the precursor lesion of esophageal adenocarcinoma.

Methods

Data from the prospective Netherlands Cohort Study were used. This cohort study was initiated in 1986, when 120,852 subjects aged 55-69 years completed a questionnaire on dietary habits and lifestyle, and provided toenail clippings for the determination of baseline selenium status. After 16.3 years of follow-up, 253 BE cases (identified through linkage with the nationwide Dutch pathology registry) and 2039 subcohort members were available for case-cohort analysis. Cox proportional hazards models were used to calculate incidence rate ratios (RR).

Results

The multivariable adjusted RR for the highest versus the lowest quartile of toenail selenium was 1.06 (95% CI 0.71-1.57). No dose-response trend was seen (p -trend=0.99). No association was found in subgroups defined by sex, smoking status, body mass index (BMI), or intake of antioxidants. For BE cases that later progressed to high-grade dysplasia or adenocarcinoma, the RR for a selenium level above the median vs. below the median was 0.64 (95% CI 0.24-1.76).

Conclusions

In this large prospective cohort study, we found no evidence of an association between selenium and risk of BE.

INTRODUCTION

Barrett's esophagus (BE) is a disease of the distal esophagus. Normally, the esophagus is lined with stratified squamous epithelium, which is replaced by a single layer of columnar epithelium (metaplasia) in BE patients. Diagnostic criteria for BE differ across the world. In the USA, the presence of goblet cells (indicating specialized intestinal metaplasia, SIM) is required for the diagnosis of BE,¹ while in the UK any type of metaplasia is sufficient for the diagnosis of BE.² In the Netherlands, both definitions have been used by different pathologists over time.

BE is most common in middle-aged Caucasian men³ and in the Netherlands, the incidence is rising among men and women.⁴ BE has primarily been of interest because patients are at increased risk to develop adenocarcinoma of the esophagus. The reported risk of esophageal adenocarcinoma in BE patients is highly variable between publications and was found in a recent meta-analysis to be between 4.1/1,000 and 6.1/1,000 person-years.⁵ Patients in whom high-grade dysplasia is present in the BE tissue are at highest risk of developing esophageal adenocarcinoma.⁶

Gastroesophageal reflux disease has been identified as a strong risk factor for BE, and possibly overweight or obesity are risk factors as well.⁷ Lifestyle factors, including diet, may also play a role in BE etiology,⁷⁻⁹ but little information is available on this topic.

One dietary factor of interest is selenium, a trace element, which has been investigated for its possible role in cancer etiology. Selenium is involved in various anticarcinogenic processes and may act at a number of stages in cancer development.¹⁰ Selenium is incorporated into some selenoproteins including glutathione peroxidases (GPx), which are antioxidant enzymes and protect against oxidative damage. Selenium is further involved in alteration of DNA methylation, blockage of the cell cycle, induction of apoptosis and inhibition of angiogenesis.¹⁰ Selenium has been associated with several cancers: it may protect against prostate, lung, gastric, and colorectal cancer.¹¹⁻¹³ Moreover, we recently found evidence suggestive of an inverse association with esophageal adenocarcinoma. This association was found in women, never smokers, and persons with a low intake of antioxidants.¹⁴ We identified only one study that looked into the association between selenium and BE. That study found a lower average serum selenium concentration among patients with BE than among controls, indicating a possible inverse association. Unfortunately, no measure of relative risk or multivariable adjusted results were presented. Two cross-sectional studies among BE cases in the USA found indications for an inverse association between serum selenium and markers of progression of BE.^{15,16} Further, a recent study reported promotor hypermethylation of the gene coding for the selenoprotein GPx3 in Barrett's esophagus tissue, thereby epigenetically inactivating this gene.¹⁷ We hypothesize that selenium status is inversely associated with risk of BE, if selenium is involved early in the process of carcinogenesis.

The selenium content of our food may vary considerably between varieties of the same type of food, depending on the soil where the food was grown.¹⁸ For that reason, selenium intake from diet cannot be estimated reliably using questionnaires. Selenium status is therefore often measured using biomarkers. In this study, we used toenails as a biomarker of selenium intake. Toenails are a suitable biomarker, because they reflect the intake of selenium for a period up to one year.^{19,20}

Within the prospective Netherlands Cohort Study on diet and cancer, we investigated whether toenail selenium levels were inversely associated with risk of BE.

MATERIALS AND METHODS

Study design and participants

The prospective Netherlands Cohort Study on diet and cancer was started in September 1986, when 58,279 Dutch men and 62,573 women aged 55-69 were enrolled. The subjects were selected at random from 204 Dutch municipal registries. All cohort members completed a self-administered questionnaire and were requested to provide toenail clippings at baseline. The study was described in detail before.²¹

A case-cohort approach²² was used for data processing and analysis for efficiency; case subjects were derived from the entire cohort, and the number of person-years at risk for the entire cohort was estimated from a subcohort of 3,500 subjects who were selected at random from the full cohort at baseline.

The subcohort was followed-up for vital status and after 16.3 years (September 1986 to December 2002), only one male subcohort member was lost to follow-up. We excluded subcohort members who reported having prevalent BE or cancer (other than skin cancer) at baseline (Figure 3.1).

The Medical Ethics Committee of Maastricht University, the Netherlands, has approved the study.

Follow-up for BE incidence

Incident BE cases in the total cohort were detected by computerized record linkage to the nationwide network and registry of histopathology and cytopathology in the Netherlands (PALGA).²³ This network was founded in 1971, and an increasing number of laboratories joined PALGA such that it has a nationwide coverage since 1991. Due to this incomplete coverage, we may have missed BE cases diagnosed between baseline (1986) and 1991. We calculated how many cases we may have missed by multiplying the mean number of cases per year during the period a lab was connected by the number of years the lab was not connected to PALGA. This was done for each lab. This way we calculated that we may have missed approximately 3% of BE cases. At each of the 64 pathology laboratories in the Netherlands, excerpts of all pathology reports are

generated automatically. Each excerpt contains a so-called PALGA diagnosis that describes topography, morphology, function, procedure, and disease. These excerpts are transferred to a central databank.²³ The linkage with PALGA was carried out for 16.3 years of follow-up.²⁴ All links were then manually checked for false-positives.

Thereafter, one pathologist (A.L.C.D.) and one pathologist in training (C.J.R.H.), who were blinded to the exposure status (i.e. the selenium status) of the cases, reviewed the excerpts of all pathology records to extract information on the initial date of diagnosis of BE, the type of metaplasia, and the degree of dysplasia. Excluded were BE cases with an uncertain diagnosis, or a diagnosis specifying the presence of only non-intestinal type metaplasia. Also excluded were cases with a diagnosis of esophageal or gastric cancer before or less than a half year after the diagnosis of BE, and BE cases that were prevalent at baseline (Figure 3.1).

Figure 3.1 Flow diagram of subcohort members and Barrett's esophagus cases on whom the analyses were based. ^a For reasons of efficiency, toenail material of Barrett's esophagus cases were not sent to laboratory for instrumental neutron activation analysis for determination of selenium levels, if they had incomplete or inconsistent questionnaire data. Therefore, this exclusion criterion did not anymore lead to exclusion of cases at this stage.

Two definitions of BE were used: our primary case definition included only subjects with esophageal SIM. The secondary case definition included subjects (a) fulfilling the primary case definition or (b) with a pathology report stating 'Barrett's', without a description of the type of metaplasia. Furthermore, BE cases may progress to high-grade dysplasia or esophageal adenocarcinoma. We followed the BE cases until December 31, 2002 and we identified the subgroup of BE cases who showed one of these complications. For analyses of BE cases who progressed to esophageal adenocarcinoma, we selected only BE cases in whom the adenocarcinoma was diagnosed more than a half year after the diagnosis of BE. This time lag was chosen to be more sure that this was a new diagnosis of cancer that followed the BE.

Exposure data

In our cohort, about 75% of the subjects provided toenail clippings. Toenail selenium determinations were carried out by the Reactor Institute Delft (Delft University of Technology, Delft, the Netherlands). The determination was based on instrumental neutron activation analysis of the ^{77}mSe isotope (half-life 17.5 s). Each sample went through 6 cycles of 17 s irradiation at a thermal neutron flux of $3 \times 10^{16} \text{ m}^{-2}\text{s}^{-1}$, 3 s decay, and 17 s counting at 1 cm from a 40% germanium detector. This method, its accuracy and precision, and the use in the Netherlands Cohort Study have been described in more detail previously.²⁵⁻²⁸

In 1992, the toenail selenium determinations for the subcohort members were carried out (for the purpose of analysis of the association between selenium and risk of several cancers). In 2008, the toenail selenium determinations were carried out for the BE cases. In 1992, the 'SBP' facility was used for instrumental neutron activation analysis, and since 1996 the 'CAFIA' facility has been used. To assess the comparability of these two methods, the toenail selenium levels for 40 subcohort members were assessed in 1996 with the 'CAFIA' facility in addition to the original assessment with the 'SBP' facility.²⁵ The mean (SD) selenium level assessed by the 'CAFIA' facility [0.552 (0.05) $\mu\text{g/g}$] was comparable with mean selenium levels assessed by the 'SBP' facility [0.551 (0.04) $\mu\text{g/g}$] for these subjects. The Pearson correlation coefficient between toenail selenium levels assessed by the 'CAFIA' facility and those estimated by the 'SBP' facility was 0.95 ($p < 0.01$).²⁵ It was concluded that both methods were comparable.

In 1992, all toenail clippings provided were sent to the Reactor Institute Delft for selenium determination. This determination however, yields unreliable results if the nails weigh $< 10 \text{ mg}$ and these measurements were thus excluded. In 2007, we discovered that toenail clippings can also be used as a source of DNA.²⁹ We therefore separated and saved 10-20 mg of toenail clippings from the BE cases. If afterwards, more than 10 mg of toenail clippings were left, we sent them to the Reactor Institute Delft for selenium determination. In case there were problems with the determination of toenail selenium the subject was excluded (Figure 3.1).

The self-administered questionnaire, which was filled out by all cohort members at baseline, consisted of a 150-item food frequency questionnaire (FFQ) and other questions, e.g. on smoking, education, height, weight, and use of medication. The FFQ asked about habitual consumption in the year before the start of the study. We calculated mean daily nutrient intakes using the Dutch food-composition table³⁰. The questionnaire data were key entered and processed in a standardized manner, blinded with respect to cases/subcohort status to minimize observer bias in coding and interpretation of the data.

Statistical analysis

To evaluate the potential influence of prediagnostic BE at baseline on toenail selenium levels, cases were categorized according to the year of follow-up in which they were diagnosed. The mean toenail selenium levels of the cases according to the year of follow-up were compared and differences were tested using a t-test. For the t-test, selenium levels were ln-transformed to normalize the distribution. For the case-cohort analysis, toenail selenium levels were categorized into quartiles according to the distribution in the subcohort. For continuous analysis, a 0.06 µg/g increment in toenail selenium was chosen. This is equal to the average size of the two central quartiles.

We excluded subjects who had inconsistent or incomplete dietary questionnaire data, because dietary data were needed as potential confounders and in subgroup analyses.³¹ Subjects with missing data on the confounders were also excluded (Figure 3.1).

The following variables were considered confounders and included in the multivariable regression model: age, sex, cigarette smoking (current yes/no, number of cigarettes/day, and number of smoking years), alcohol consumption (g/day), and body mass index (kg/m²). The following variables were considered potential confounders, but were not included in the models because they did not change the incidence rate ratio (RR) by >5%: highest level of education, family history of esophageal or gastric cancer, reported long-term (>0.5 years) use of nonsteroidal anti-inflammatory drugs or aspirin, or lower esophageal sphincter relaxing medication,³² non-occupational physical activity, daily intakes of the antioxidants vitamin C, vitamin E, α-carotene, β-carotene, β-cryptoxanthin, lycopene, and lutein/ zeaxanthin. Figure 3.1 shows that complete data were available for 2039 subcohort members, 253 BE cases fulfilling the primary case definition and 346 BE cases fulfilling the secondary case definition.

Multivariable adjusted RR and corresponding 95% confidence intervals (CI) were estimated using Cox proportional hazards models³³ in Stata 9.2 (StataCorp, College Station, Texas, USA). Standard errors were estimated using the robust Huber-White sandwich estimator to account for additional variance introduced by sampling from the cohort. This method is equivalent to the variance-covariance estimator by Barlow.³⁴ We tested the proportional hazards assumption using the scaled Schoenfeld residuals.³⁵ Tests for dose-response trends were assessed by fitting ordinal exposure

variables as continuous terms. Two-sided P values are reported throughout the article. The significance level α was set at 0.05.

We investigated possible interactions between toenail selenium status and sex, cigarette smoking status, BMI, and intakes of several antioxidants by estimating RR in strata of these exposures. The p value for interaction was assessed by including a cross-product term in the model. To evaluate potential differences in the association during early and late follow-up, we stratified our analysis by follow-up period. Finally, we analyzed the subgroup of BE cases that progressed to high-grade dysplasia and/or esophageal adenocarcinoma during follow-up until 2002, as this is the group most relevant with respect to disease burden. Because of the low number of cases in this subgroup ($n=21$), we used two instead of four exposure categories: above and below the median selenium concentration.

RESULTS

Descriptives

Table 3.1 shows the mean toenail selenium levels of BE cases, stratified by two-year follow-up periods in which they were diagnosed. The toenail selenium levels in the first two follow-up years were lower than in later follow-up years, and this difference was borderline statistically significant ($p=0.07$) for BE cases with SIM. As this difference may indicate an effect of prediagnostic disease on toenail selenium levels, we decided to exclude the first two years of follow-up from the analysis to prevent bias.

Table 3.1 Toenail selenium levels ($\mu\text{g/g}$) in Barrett's esophagus cases according to sex and time between baseline and diagnosis; Netherlands Cohort Study (1986-2002, $n = 120,852$).

Cases ^a	Barrett's esophagus							
	SIM				SIM or unknown metaplasia			
	Toenail selenium level ($\mu\text{g/g}$)				Toenail selenium level ($\mu\text{g/g}$)			
	No. cases	mean	SD	p^b	No. cases	mean	SD	p^b
All cases	285	0.563	0.097		397	0.562	0.112	
Men	163	0.556	0.100		226	0.558	0.127	
Women	122	0.571	0.093		171	0.568	0.087	
Follow-up year in which case was diagnosed								
0-2	5	0.488	0.047	0.07	10	0.536	0.099	0.39
>2-4	17	0.531	0.086		31	0.566	0.210	
>4-6	17	0.540	0.087		28	0.553	0.091	
>6-8	38	0.557	0.058		54	0.552	0.068	
>8-10	50	0.573	0.113		71	0.565	0.102	
>10-12	55	0.555	0.096		74	0.561	0.120	
>12-14	54	0.585	0.107		69	0.572	0.103	
>14-17	49	0.568	0.099		60	0.565	0.094	

^a Mean (SD) selenium levels in subcohort members were 0.547 (0.126) $\mu\text{g/g}$ for men ($n=1212$) and 0.575 (0.109) $\mu\text{g/g}$ for women ($n=1244$); ^b T-test of mean toenail selenium levels (ln-transformed) for cases diagnosed in the first 2 years of follow-up versus levels for cases diagnosed during the remainder of follow-up years.

Characteristics of the subcohort and BE cases are described in Table 3.2. Subcohort members had a median toenail selenium level of 0.553 $\mu\text{g/g}$, with lower levels in men (median 0.539 $\mu\text{g/g}$) than in women (0.564 $\mu\text{g/g}$). The median toenail selenium levels in the two BE case groups were comparable (0.552 and 0.553 $\mu\text{g/g}$). With respect to other characteristics, there were more men and former smokers among the cases, when compared with the subcohort. Furthermore, cases had somewhat lower intakes of carotenoids, and were more likely to be long-term users of non-steroidal anti-inflammatory drugs/aspirin and lower esophageal sphincter relaxing medication.

Main analysis

Table 3.3 presents multivariable adjusted RR of BE according to toenail selenium levels. The RR of BE with SIM was 1.06 (95% CI: 0.71, 1.57) for the highest versus the lowest quartile of toenail selenium, and no dose-response trend was observed (p trend=0.99). Multivariable analyses stratified by sex showed some differences in associations for men (RR quartile 4 vs. quartile 1 = 1.31, 95% CI: 0.78, 2.21) and women (RR quartile 4 vs. quartile 1=0.85, 95% CI: 0.46, 1.57), but the interaction between toenail selenium and sex was not statistically significant (p interaction=0.18). The results were all comparable to the age- and sex-adjusted results (Table 3.3). When we used the less stringent secondary case definition of BE, the results were very similar to those based on the primary case definition (Table 3.3).

Interaction and subgroup analyses

When analyses were performed in strata of smoking status, body mass index, or daily intake of antioxidants, no statistically significant or important differences in associations were observed between strata, and the RR were all around unity. Again, results were similar for the two BE case groups (see Supplemental Table 3.1). Analyses stratified by follow-up period (early vs. late follow-up) showed no statistically significant associations or dose-response trends in either period (data not shown).

An inverse association was found between toenail selenium and risk of BE that progressed to high-grade dysplasia and/or adenocarcinoma (Table 3.4). The multivariable RR for a toenail selenium level above the median vs. below the median was 0.64, although the confidence interval was relatively wide (95% CI: 0.24, 1.76), due to the low case number in this analysis ($n=21$).

Table 3.2 Characteristics of cases and subcohort members in the Netherlands Cohort Study (1986-2002, n=120,852).

Characteristic	Subcohort	Barrett's esophagus cases	
	(n=2039) ^a	SIM (n=253) ^a	SIM or unknown metaplasia (n=346) ^a
	Median (IQR)	Median (IQR)	Median (IQR)
Toenail selenium level (µg/g)			
total	0.553 (0.498-0.613)	0.552 (0.501-0.615)	0.553 (0.500-0.607)
men	0.539 (0.483-0.602)	0.551 (0.495-0.615)	0.544 (0.492-0.605)
women	0.564 (0.514-0.623)	0.555 (0.508-0.622)	0.565 (0.509-0.613)
	Mean (SD) ^b	Mean (SD) ^b	Mean (SD) ^b
Age at baseline (years)	61.2 (4.2)	60.8 (4.2)	61.1 (4.1)
Men (%)	49	59	58
Cigarette smoking status			
never smoker (%)	38	30	31
former smoker (%)	37	51	48
current smoker (%)	25	19	21
Ever cigarette smokers:			
Frequency of cigarette smoking (n/day)	15.2 (10.3)	16.6 (11.6)	16.5 (11.1)
Duration of cigarette smoking (years)	31.0 (12.2)	30.4 (11.8)	30.7 (12.0)
Ethanol intake (g/day)	10.2 (14.3)	10.8 (15.0)	10.8 (15.0)
Body mass index (kg/m ²)	25.0 (3.1)	25.3 (2.9)	25.3 (2.8)
Non-occupational physical activity (min/day)	73 (58)	69 (53)	70 (55)
Highest level of education			
primary (%)	27	27	30
lower vocational (%)	22	20	20
secondary and medium vocational (%)	37	36	35
university and higher vocational (%)	14	17	15
Vitamin C intake (mg/day)	104 (42)	101 (44)	100 (43)
Vitamin E intake (mg/day)	14 (6)	14 (6)	13 (6)
α-carotene intake (µg/day)	711 (588)	670 (502)	670 (512)
β-carotene intake (µg/day)	3001 (1589)	2821 (1382)	2805 (1398)
β-cryptoxanthin intake (µg/day)	181 (165)	177 (167)	176 (166)
Lycopene intake (µg/day)	1214 (1773)	1203 (2469)	1125 (2202)
Lutein/zeaxanthin intake (µg/day)	2518 (1079)	2353 (904)	2341 (925)
Family history of esophageal or gastric cancer (%)	8	8	8
Use of non-steroidal anti-inflammatory drugs and aspirin (%) ^c	7	11	10
Use of lower esophageal sphincter relaxing medication (%) ^c	14	19	20

IQR, interquartile range. ^a Presented are the number of subcohort members or cases with complete data on toenail selenium level, age, sex, cigarette smoking (current yes/no, number of cigarettes smoked daily, number of smoking years), alcohol consumption and body mass index. Subcohort members and cases with incomplete or inconsistent questionnaire data are excluded; ^b For categorical variables a percentage is presented; ^c Self reported use during more than 0.5 year.

Table 3.3 Incidence rate ratios of Barrett's esophagus according to toenail selenium levels; Netherlands Cohort Study (1986-2002, n=120,852).

	Subcohort		Barrett's esophagus							
			SIM				SIM or unknown metaplasia			
	Median (µg/g)	person time at risk (years)	no.	RR	Age- and sex-adjusted 95% CI	RR	Age- and sex-adjusted 95% CI	no.	RR	Age- and sex-adjusted 95% CI
All subjects										
Quartiles of toenail selenium (boundaries in µg/g)										
1 (≤0.498)	0.458	6170	59	1	reference	1	reference	83	1	reference
2 (≤0.552)	0.525	6548	69	1.15	0.79, 1.67	1.08	0.74, 1.58	90	1.06	0.77, 1.48
3 (≤0.613)	0.580	6659	59	0.99	0.67, 1.45	0.92	0.62, 1.37	89	1.06	0.76, 1.47
4 (>0.613)	0.667	6527	66	1.15	0.78, 1.68	1.06	0.71, 1.57	84	1.03	0.74, 1.44
p trend ^b					0.69		0.99			0.87
continuous (0.06 µg/g increment) ^c		25903	253	1.01	0.96, 1.07	1.00	0.94, 1.06	346	1.01	0.96, 1.07
					p interaction ^d 0.17		p interaction 0.18			p interaction 0.06
Men										
Quartiles of toenail selenium (boundaries in µg/g)										
1 (≤0.498)	0.458	3658	39	1	reference	1	reference	55	1	reference
2 (≤0.552)	0.525	3078	37	1.10	0.68, 1.79	1.06	0.64, 1.76	54	1.14	0.75, 1.73
3 (≤0.613)	0.580	2830	34	1.10	0.67, 1.80	1.07	0.64, 1.78	44	1.02	0.66, 1.57
4 (>0.613)	0.667	2596	38	1.35	0.84, 2.19	1.31	0.78, 2.21	48	1.21	0.79, 1.86
p trend					0.26		0.33			0.50
continuous (0.06 µg/g increment)		12162	148	1.03	0.98, 1.09	1.03	0.97, 1.09	201	1.04	0.98, 1.09
Women										
Quartiles of toenail selenium (boundaries in µg/g)										
1 (≤0.498)	0.458	2512	20	1	reference	1	reference	28	1	reference
2 (≤0.552)	0.525	3469	32	1.16	0.64, 2.09	1.14	0.63, 2.08	36	0.93	0.55, 1.58
3 (≤0.613)	0.580	3829	25	0.82	0.44, 1.51	0.77	0.41, 1.43	45	1.05	0.63, 1.75
4 (>0.613)	0.667	3931	28	0.89	0.49, 1.63	0.85	0.46, 1.57	36	0.82	0.48, 1.39
p trend					0.42		0.33			0.55
continuous (0.06 µg/g increment)		13742	105	0.95	0.85, 1.06	0.94	0.84, 1.05	145	0.94	0.86, 1.03
										0.85, 1.02

^a Adjusted for age (years), sex, cigarette smoking (current smoking status (yes/no), frequency (number of cigarettes/day), and duration (years)), alcohol consumption (g/day), body mass index (kg/m²); ^b Tests for dose-response trends were assessed by fitting ordinal variables as continuous terms in the Cox proportional hazard model;

^c The 0.06 µg/g increment for continuous analyses is equal to the average size of the two central quartiles; ^d p-value for interaction between sex and toenail selenium level, based on cross product term in the Cox proportional hazard model.

Table 3.4 Incidence rate ratios of Barrett's esophagus cases who developed high grade dysplasia or adenocarcinoma according to toenail selenium levels; Netherlands Cohort Study (1986-2002, n=120,852).

		Barrett's esophagus with high grade dysplasia and/or adenocarcinoma					
		Subcohort	Age- and sex-adjusted			Multivariable adjusted ^a	
	Median within category $\mu\text{g/g}$	person time at risk (years)	no. cases	RR	95% CI	RR	95% CI
Categories of toenail selenium, based on a median split (boundary in $\mu\text{g/g}$)							
1 (≤ 0.552)	0.497	12717	13	1	reference	1	reference
2 (> 0.552)	0.615	13185	8	0.63	0.26, 1.56	0.64	0.24, 1.76
Continuous (0.06 $\mu\text{g/g}$ Increment) ^b		25903	21	1.10	0.99, 1.22	1.11	0.99, 1.24

^a adjusted for age (years), sex, cigarette smoking (current smoking status (yes/no), frequency (number of cigarettes/day), and duration (years)), alcohol consumption (g/day), body mass index (kg/m^2); ^b The RRs of the continuous analyses are influenced by one case with a very high toenail selenium level (1.612 $\mu\text{g/g}$). When this case was excluded, the age- and sex-adjusted RR was 0.96 (95% CI 0.79-1.17) and the multivariable adjusted RR was 0.96 (95% CI 0.77-1.20).

DISCUSSION

To our knowledge, this is the second investigation, and first cohort study, into the possible role of selenium in the etiology of BE, a precursor lesion of esophageal adenocarcinoma. The results from this prospective cohort study do not support our hypothesis that an inverse association might exist. Neither did we find evidence of an association in subgroups defined by sex, smoking status, BMI, intake of antioxidants or follow-up period.

The Dutch population that was used in this study, has a low to moderate toenail selenium level (mean: 0.564 $\mu\text{g/g}$ in the subcohort) when compared with mean levels found in other populations (general population or control subjects): China (Sichuan province) 0.211 $\mu\text{g/g}$,³⁶ Finland 0.47 $\mu\text{g/g}$,³⁷ USA 0.83-0.92 $\mu\text{g/g}$,³⁸ Colombia 0.945 $\mu\text{g/g}$,³⁹ and USA (South Dakota/Wyoming) 1.517 $\mu\text{g/g}$.¹⁹

In the Netherlands, both the USA and UK case definitions of BE may have been used. The use of these definitions may have changed over time and different pathologists may have used different definitions.

The first strength of our study is its prospective character, which brings the advantage of measuring the selenium status before the diagnosis of BE. This method makes it unlikely that the exposure was changed due to the disease or knowledge of the diagnosis, and therefore it lowers the chance of reversed causation. A second strength is the size of the study. This study had 80% power to detect an RR of 0.63 for

all BE cases and an RR of 0.59 for BE cases with SIM.⁴⁰ Thirdly, we had the possibility to adjust for confounding by several lifestyle factors. A fourth strength is the use of toenail clippings for the measurement of selenium exposure, because selenium levels in clippings from all toes represent an exposure period of up to one year. Measuring long-term exposure is important as it more likely represents the exposure in the etiologically relevant time window compared with a measure of short-term exposure.

A limitation of our study is the lack of information about gastroesophageal reflux disease and medications related to this disease. In the baseline questionnaire one question concerned the long-term use of medications and the disease for which these were prescribed. However, we believe that the reported use of reflux medication and antacids, and presence of gastroesophageal reflux disease were substantially lower than the actual frequency. This prohibited us to use this information in the analysis. Consequently, we were not able to evaluate if gastroesophageal reflux disease is a confounder or intermediate factor in the relation between selenium and BE. We therefore suggest that this study is replicated in a population for which information on gastroesophageal reflux is available, to evaluate its possible role as confounder or intermediate factor. A second limitation is the single measurement of the exposure. Selenium exposure may have changed during follow-up due to changed selenium levels in foods or changed dietary habits. Third, we did not have the opportunity to verify the absence of BE in the subcohort members who were not diagnosed with BE. BE usually occurs in patients with gastroesophageal reflux disease, but it has also been described in asymptomatic individuals, who are therefore not diagnosed with the disease. Three studies investigated the prevalence of BE among asymptomatic individuals in the USA and reported prevalences ranging from 1% to 25%.⁴¹⁻⁴³ However, in our cohort study, any undiagnosed BE cases would most likely not have influenced the RR. This is because the imperfect sensitivity (*i.e.* false-negatives) is combined with good specificity (*i.e.* few false positives), and the chance of diagnosis is likely independent of selenium status. The misclassification of the disease status then is non-differential and will not influence the RR.⁴⁴ Still, a consequence of undiagnosed cases is a reduced power to detect an existing association. A fourth limitation, related to the third, is caused by the incomplete coverage of the Netherlands by PALGA before 1991. There may have been some BE cases that were diagnosed in laboratories when these had not yet joined PALGA. If these cases were not followed-up, we missed their diagnosis. If they were followed-up after the laboratory joined PALGA, the incidence date we registered is more recent than the true incidence date. We assessed that we may have missed 3% of the BE cases in our cohort at most.

If, in line with our null findings there is truly no association between toenail selenium and the risk of BE, this does not preclude a possible association between selenium and progression of BE to high-grade dysplasia or adenocarcinoma. We observed an RR well below unity for subjects with a toenail selenium level above the median in those BE cases who progressed to high-grade dysplasia and/or esophageal adenocarcinoma. This RR, however, was based on few cases and was not statistically

significant. In a previous analysis we found indications for an inverse association between selenium and risk of esophageal adenocarcinoma in some subgroups: women, never smokers, and low antioxidant consumers.¹⁴ These above-mentioned observations are in agreement with observations by two other studies that found indications for a role of selenium in later stages of the carcinogenesis of esophageal adenocarcinoma.^{15,16} Selenium might be an interesting preventive agent for progression to esophageal adenocarcinoma in patients with BE, but this requires a larger body of evidence.

In conclusion, no evidence of an inverse association between toenail selenium and risk of BE was found in this study. An inverse association might exist for BE cases that progress to high-grade dysplasia and/or esophageal adenocarcinoma, but results from other studies are needed before any firm conclusions can be drawn. Preferably, future studies should have a prospective character and should have the possibility to investigate the role of gastroesophageal reflux in this association. Also, it would be informative to study the relation between selenium and risk of BE in a population with selenium levels that are lower or higher compared with our study population.

Supplemental Table 3.1 Association ^a between toenail selenium levels and risk of Barrett's esophagus stratified by cigarette smoking status, body mass index and intake levels of antioxidants; Netherlands Cohort Study (1986-2002, n=120,852)

	Subcohort	Barrett's esophagus					
	person time at risk (years)	SIM			SIM or unknown metaplasia		
		no. cases	RR ^b	95% CI	no. cases	RR ^b	95% CI
Cigarette smoking status							
Never smoker	10157	77	0.98	0.87, 1.10	108	0.95	0.86, 1.05
Former smoker	9358	128	1.00	0.92, 1.08	165	1.00	0.93, 1.08
Current smoker	6389	48	1.07	0.88, 1.29	73	1.14	0.91, 1.43
		p interaction		0.78	p interaction		0.35
Body mass index (kg/m²)							
< 25	13930	131	1.01	0.96, 1.07	178	1.01	0.95, 1.06
≥ 25	11973	122	0.96	0.83, 1.10	168	0.99	0.86, 1.14
		p interaction		0.73	p interaction		0.79
Antioxidant intake							
Vitamin C intake (mg/d)							
low (≤ 97)	12700	139	1.01	0.95, 1.09	189	1.01	0.94, 1.07
high (> 97)	13204	114	0.99	0.90, 1.08	157	1.01	0.91, 1.12
		p interaction		0.76	p interaction		0.90
Vitamin E intake (mg/d)							
low (≤ 12)	12901	113	1.01	0.90, 1.13	166	1.06	0.95, 1.19
high (> 12)	13002	140	0.99	0.92, 1.07	180	0.96	0.89, 1.04
		p interaction		0.78	p interaction		0.16
α-carotene intake (μg/d)							
low (≤ 573)	12708	132	1.04	0.93, 1.16	183	1.03	0.94, 1.14
high (> 573)	13195	121	0.97	0.89, 1.06	163	0.99	0.90, 1.08
		p interaction		0.37	p interaction		0.46
β-carotene intake (μg/d)							
low (≤ 2680)	12817	139	1.01	0.95, 1.08	196	1.01	0.95, 1.07
high (> 2680)	13086	114	0.97	0.88, 1.08	150	1.00	0.88, 1.12
		p interaction		0.62	p interaction		0.78
β-cryptoxanthin intake (μg/d)							
low (≤ 130)	12669	129	1.01	0.91, 1.13	176	1.03	0.92, 1.15
high (> 130)	13234	124	0.99	0.92, 1.07	170	0.99	0.92, 1.06
		p interaction		0.60	p interaction		0.48
Lycopene intake (μg/d)							
low (≤ 823)	12857	141	0.98	0.89, 1.07	203	0.96	0.89, 1.04
high (> 823)	13046	112	1.04	0.94, 1.14	143	1.08	0.97, 1.20
		p interaction		0.46	p interaction		0.13
Lutein/Zeaxanthin intake (μg/d)							
low (≤ 2360)	12876	146	1.00	0.92, 1.08	198	0.99	0.92, 1.07
high (> 2360)	13027	107	1.01	0.91, 1.12	148	1.02	0.92, 1.14
		p interaction		0.66	p interaction		0.60

^a Adjusted for age (years), sex, cigarette smoking (current smoking status (yes/no), frequency (number of cigarettes/day), and duration (years)), alcohol consumption (g/day), body mass index (kg/m²), if applicable;

^b RR per 0.06 μg/g increment of toenail selenium. This increment is equal to the average size of the two central quartiles; ^c p-value for interaction between sex and toenail selenium level, based on cross product term in the Cox proportional hazard model.

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4

Cancer incidence and cause-specific mortality in a population-based cohort of patients with Barrett's esophagus

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ABSTRACT

Objective

Barrett's esophagus (BE) is associated with an increased risk of esophageal adenocarcinoma, but evidence with respect to incidence of other cancers and overall mortality is inconsistent.

Methods

The Netherlands Cohort Study was initiated in 1986, when 120,852 participants aged 55-69 years at baseline were included. Until December 2002, 626 incident BE cases (excluding non-intestinal metaplasia) were identified by record linkage with the nationwide Pathology Registry. Cancer and mortality follow-up of this cohort of BE cases was established by record linkage to the Netherlands Cancer Registry and Statistics Netherlands. The expected number of cases was calculated using national cancer incidence and mortality data.

Results

After a median follow-up of 5.7 years of the BE cohort, 13 esophageal and 5 gastric cancer cases were recorded. The Observed/Expected (O/E) ratios for esophageal and gastric cancer were 10.0 (95% Confidence Interval (CI), 5.3-17.1) and 1.8 (95% CI, 0.6-4.2), respectively.

Total cancer incidence (excluding esophageal and gastric cancer) in the BE cohort was nearly statistically significantly increased: O/E-ratio 1.3 (95% CI, 1.0-1.6). Of the subtypes, small intestinal and pancreatic cancer incidence were increased, but not statistically significantly after exclusion of the first six months of follow-up.

During follow-up 225 BE cases died. Mortality of all causes (excluding esophageal and gastric cancer) was not increased: O/E-ratio 1.0 (95% CI, 0.9-1.2), neither was mortality according to main causes of death.

Conclusion

In this population-based cohort of BE cases esophageal cancer incidence was increased. Total cancer incidence and overall mortality were not increased.

INTRODUCTION

Barrett's esophagus (BE) is an abnormal appearing distal esophageal lining with replacement of the normal squamous epithelium by specialized or intestinal columnar epithelium.¹ BE is thought to be a pre-cancerous condition and patients with BE have an increased risk to develop esophageal adenocarcinoma.² Although many studies have investigated the risk of esophageal adenocarcinoma in patients with BE,^{3,4} most follow-up studies are small and have been conducted in selected patient series. Larger studies with complete follow-up and a strict definition of BE have found lower risks of esophageal adenocarcinoma compared to studies with a smaller study-size.^{4,5} Incidence of gastric cancer was also increased in some studies, possibly due to misclassification of junctional tumors.³ The number of studies examining incidence rates of other cancers and mortality is limited. There are indications that colorectal cancer risk is increased in patients with BE, but this may be due to selected patient series and detection bias.^{6,7}

Several studies have observed increased mortality rates among BE cases.⁸⁻¹¹ However, the only population-based cohort that studied mortality did not observe an increased risk.¹²

We therefore decided to study cancer incidence and overall mortality in the population-based Netherlands Cohort Study on Diet and Cancer. By record-linkage to the Netherlands nationwide pathology registry, cases of BE in the cohort were identified. By follow-up of these cases we were able to study whether cancer incidence and cause-specific mortality were increased in this population-based cohort of BE cases.

PATIENTS AND METHODS

Study design and subjects

The Netherlands Cohort Study was started in September 1986, when 58,279 Dutch men and 62,573 women aged 55-69 years were enrolled. The subjects were selected at random from 204 Dutch municipal population registries. All cohort members completed a self-administered questionnaire. The study design has been described in detail before.¹³

Incident BE cases in the total cohort were identified by computerized record-linkage to the nationwide network and registry of histopathology and cytopathology in the Netherlands (PALGA) using the search term "Barrett" or the search terms "metaplasia" combined with "esophagus".⁵ The linkage was carried out for the period until 31 December 2002, using the linkage protocol that we described before.¹⁴ All links were checked for false-positives (see Figure 4.1). Thereafter, one pathologist (A.L.C.D.) and one pathologist in training (C.J.R.H.) reviewed the available summary and

conclusion of the pathology records from PALGA to extract information on the date of diagnosis of BE, the type of metaplasia, and the presence and degree of dysplasia.

Excluded were BE cases with an uncertain diagnosis or with a diagnosis specifying the presence of only non-intestinal metaplasia. Also excluded were BE cases with prevalent cancer (self-reported) or prevalent BE (if known) in September 1986. To eliminate the possibility that the diagnosis of BE was related to (preclinical) symptoms of esophageal or gastric cancer, we excluded BE cases with a diagnosis of esophageal or gastric cancer before or less than a half year after the diagnosis of BE (leaving 626 cases). This constituted a population-based cohort of BE cases. The Medical Ethics Committee of Maastricht University, the Netherlands, has approved the study.

Figure 4.1 Flow diagram of Barrett's esophagus (BE) cases on whom the analyses were based.

Follow-up for cancer incidence and cause-specific mortality

For the analyses with respect to esophageal and gastric cancer incidence, total cancer incidence, and overall mortality, three different datasets were constructed from the BE cohort. Because we had excluded all esophageal or gastric cancer in the first half year of follow-up after BE diagnosis, the follow-up for esophageal and gastric cancer started a half year after BE diagnosis (leaving 605 patients at risk). For the follow-up of cancer incidence, BE cases with cancer (other than non-melanoma skin cancer) diagnosed before the BE diagnosis were excluded (leaving 561 patients). No additional exclusion criterion was necessary for the dataset for the mortality follow-up (626 patients).

Follow-up for vital status was established by record linkage with the Central Bureau of Genealogy and the automated municipal population registries. Follow-up for vital status was complete for all 626 members of the Barrett's cohort at 1 May 2005.

Follow-up for cancer incidence in BE cases was performed by record linkage to the Netherlands Cancer Registry and PALGA.¹⁴ For the cancer incidence analyses, follow-up data were available until 31 December 2003. In this analysis only the first occurrence of a primary cancer was counted.

We were able to obtain the cause of death from Statistics Netherlands (until 1 May 2005). Causes of death were coded in the International Classification of Diseases (ICD)-9 until December 1995 and ICD-10 from January 1996. Only the primary cause of death was used in the analysis.

Statistical analysis

Cancer incidence in the Barrett's cohort was compared with the incidence in the Dutch population. Time at risk began at diagnosis of BE (or a half year after BE diagnosis in the esophageal and gastric cancer analysis) and ended on the date of cancer diagnosis, the date of death, or the last date of the follow-up for cancer incidence (31 December 2003), whichever occurred first. The expected numbers of cancer, taking into account the person-years of observation in the Barrett's cohort, were computed with the use of sex-, age-, and calendar year – specific cancer incidence rates from the Eindhoven Cancer Registry up to 1990 or,¹⁵ for the period after 1990, from the Netherlands Cancer Registry.^{16,17}

Mortality in the Barrett's cohort was compared with mortality in the Dutch population. Time at risk began at diagnosis of BE and ended on the date of death or the last date of the follow-up for mortality (1 May 2005), whichever occurred first. For the mortality analysis sex-, age- and calendar year -specific mortality rates were obtained from Statistics Netherlands.

The Observed/Expected (O/E) ratios for cancer incidence and cause-specific mortality were calculated as the ratio of the observed number of cancer cases or deaths to the expected number, and the 95% confidence intervals (CI) were calculated based on the Poisson distribution.¹⁸ The absolute excess risk of cancer was calculated by subtracting the expected number of cases from the observed number, divided by

the person-years at risk. All analyses were conducted using a statistical program written in SPSS developed by the Netherlands Cancer Institute.¹⁹

RESULTS

The dataset for esophageal and gastric cancer incidence included 605 BE cases, the dataset for incidence of other cancers included 561 persons, while the dataset for mortality follow-up counted 626 persons (see Table 4.1). The majority of the BE cases was male and the mean age at diagnosis of Barrett's esophagus was slightly higher than 71 years. In 73-74% of the BE cases, the histology was specified as intestinal metaplasia, while in the remaining cases the histology was unspecified. Dysplasia was reported not to be present in 81% of the cases, while high-grade dysplasia was rare (1%). The median follow-up duration was 5.7 years in the esophageal and gastric cancer analysis, 5.4 years in the cancer incidence analysis and 6.8 years in the mortality analysis.

Table 4.1 Baseline characteristics of cohort of patients with Barrett's esophagus for cancer incidence and mortality analysis. Netherlands Cohort Study.

Characteristic	Esophageal and gastric cancer analysis ^a		Cancer incidence analysis ^b		Mortality analysis ^c	
	n	(%)	n	(%)	n	(%)
Total	605	(100.0)	561	(100.0)	626	(100.0)
Sex						
Males	325	(53.7)	292	(52.0)	341	(54.5)
Females	280	(46.3)	269	(48.0)	285	(45.5)
Age at diagnosis of Barrett's esophagus, mean (SD) ^d	71.2	(5.3)	71.1	(5.3)	71.2	(5.3)
Incidence year						
1986-1990	72	(11.9)	68	(12.1)	75	(12.0)
1991-1994	145	(24.0)	138	(24.6)	149	(23.8)
1995-1998	214	(35.4)	193	(34.4)	222	(35.5)
1999-2002	174	(28.8)	162	(28.9)	180	(28.8)
Histology						
Intestinal metaplasia	446	(73.7)	415	(74.0)	456	(72.8)
Barrett's esophagus NOS	159	(26.3)	146	(26.0)	170	(27.2)
Dysplasia						
No dysplasia	493	(81.5)	457	(81.5)	508	(81.2)
Low-grade dysplasia	92	(15.2)	88	(15.7)	97	(15.5)
High-grade dysplasia	8	(1.3)	7	(1.2)	9	(1.4)
Unspecified dysplasia	12	(2.0)	9	(1.6)	12	(1.9)
Follow-up years ^e , mean (SD)	6.4	(3.9)	6.0	(3.9)	7.2	(4.2)

^a Cohort used for esophageal and gastric cancer analysis, start of follow-up a half year after diagnosis of Barrett's esophagus; ^b Cohort used for cancer incidence analysis, only patients who were cancer-free at diagnosis of the Barrett's esophagus; ^c Cohort used for mortality analysis; ^d SD= Standard Deviation; NOS= Not Otherwise Specified; ^e Calculated from diagnosis of Barrett's esophagus.

In the Barrett's cohort, 13 cases of esophageal cancer and 5 cases of gastric cancer were reported during follow-up (Table 4.2). The 13 esophageal cancer cases were all located in the lower third of the esophagus and the histological type was adenocarcinoma. Based on national incidence rates only 1.3 cases of esophageal cancer were expected, and the O/E-ratio was statistically significantly increased: 10.0 (95% CI: 5.3-17.1). The O/E-ratios were higher in males than in females and in cases with a BE diagnosis at age 68 to 72 years (Table 4.2). The O/E ratios were higher in cases with intestinal metaplasia, and higher in cases with unspecified dysplasia or high-grade dysplasia, although numbers were small. O/E-ratios were lower after five years of follow-up.

The O/E-ratio was 1.8 (95% CI: 0.6-4.2) for gastric cancer (Table 4.2). Four of the five observed gastric cancer cases were located in the gastric cardia; the subsite of the remaining case was unspecified. All cases of gastric cancer in the follow-up were detected in males and in cases with intestinal metaplasia.

The incidence rate of esophageal cancer was 3.7 per 1000 person-years and the absolute excess risk was 3.3 per 1000 person-years (95% CI: 1.6-5.9 per 1000 person-years). The incidence rate of gastric cancer was 1.4 per 1000 person-years and the absolute excess risk was 0.6 per 1000 person-years (95% CI: -0.3 to 2.5 per 1000 person-years) (Table 4.2).

In the dataset for cancer incidence follow-up 92 cases of invasive cancer were diagnosed during the follow-up (Table 4.3). The O/E-ratio was calculated to be 1.5 (95% CI: 1.2-1.8). Without the esophageal and gastric cancer cases the O/E-ratio was still increased but not statistically significantly anymore: O/E-ratio: 1.3 (95% CI: 1.0-1.6). The cancer incidence rate was statistically significantly increased for small intestinal cancer (O/E-ratio: 11.8; 95% CI: 1.4-42.5) and pancreatic cancer (O/E-ratio: 3.1; 95% CI: 1.0-7.2). When cases diagnosed in the first six months of follow-up were excluded, the increased risks were no longer statistically significant: O/E-ratio 6.3 (95% CI: 0.2-35.3) for small intestinal cancer and O/E-ratio 2.7 (95% CI: 0.7-6.8) for pancreatic cancer. Colorectal cancer incidence was not increased. The incidence rates of lung and prostate cancer were increased, but not statistically significantly.

The total mortality in the cohort was not statistically significantly increased (n=626 at risk) (Table 4.4). During follow-up, 225 cases died (n=213.2 expected) and the O/E-ratio was 1.1 (95% CI: 0.9-1.2). After exclusion of esophageal and gastric cancer deaths, there were 214 cases who died (n=208.5 expected). The O/E-ratio for this comparison was 1.0 (95% CI: 0.9-1.2). Mortality was not statistically significantly increased either for any of the specific causes of death (Table 4.4).

Table 4.2 Incidence of esophageal and stomach cancer in Barrett's esophagus cohort^a according to sex, age at diagnosis, histology, dysplasia and duration of follow-up (n=605). Netherlands Cohort Study, 1986-2003.

Category	n at risk	n	person-years	Observed	Expected	O/E ratio ^b	95% CI of O/E ratio	Incidence rate (per 1000 person-years)	
								Incidence rate	95% CI of AER
Esophageal cancer									
<i>Total</i>	605		3560.0	13	1.30	10.0	(5.3-17.1)	3.7	(1.6-5.9)
<i>Sex</i>									
Females	280		1760.5	2	0.33	6.0	(0.7-21.7)	1.1	(-0.1-3.9)
Males	325		1799.6	11	0.97	11.3	(5.7-20.3)	6.1	(2.5-10.4)
<i>Age at diagnosis BE</i>									
56-67 years	132		1200.0	4	0.38	10.6	(2.9-27.1)	3.3	(0.6-8.2)
68-72 years	196		1215.3	7	0.45	15.7	(6.3-32.4)	5.8	(2.0-11.5)
73+ years	277		1144.8	2	0.48	4.2	(0.5-15.1)	1.7	(-0.2-5.9)
<i>Histological Classification</i>									
Intestinal Metaplasia	446		2581.4	11	0.95	11.6	(5.8-20.7)	4.3	(1.8-7.3)
Barrett's NOS	159		978.7	2	0.35	5.7	(0.7-20.4)	2.0	(-0.1-7.0)
<i>Dysplasia</i>									
No dysplasia	493		2958.1	9	1.06	8.5	(3.9-16.2)	3.0	(1.0-5.4)
Low-grade dysplasia	92		487.6	2	0.19	10.3	(1.2-37.1)	4.1	(0.1-14.4)
High-grade dysplasia	8		44.5	1	0.02	50.5	(1.3-281.1)	22.5	(0.1-124.8)
Unspecified dysplasia	12		69.9	1	0.03	29.9	(0.8-166.3)	14.3	(-0.1-79.2)
<i>Follow-up duration^d</i>									
0-<1 years	605		578.9	2	0.19	10.4	(1.3-37.6)	3.5	(0.1-12.1)
1-4 years	549		1745.2	9	0.62	14.5	(6.6-27.6)	5.2	(2.0-9.4)
5-9 years	318		1006.6	2	0.39	5.1	(0.6-18.5)	2.0	(-0.2-6.8)
10+ years	110		229.4	-	0.10	-	--	-	-

Category	n at risk	n person-years	Observed	Expected	O/E ratio ^b	95% CI of O/E ratio	Incidence rate (per 1000 person-years)	
							Incidence rate	AER ^c 95% CI of AER
Stomach cancer								
Total	605	3560.0	5	2.78	1.8	(0.6-4.2)	1.4	0.6 (-0.3-2.5)
Sex								
Females	280	1760.5	0	0.78	-	-	-	-
Males	325	1799.6	5	2.00	2.5	(0.8-5.8)	2.8	1.7 (-0.2-5.3)
Age at diagnosis BE								
56-67 years	132	1200.0	2	0.77	2.6	(0.3-9.4)	1.7	1.0 (-0.4-5.4)
68-72 years	196	1215.3	2	0.97	2.1	(0.3-7.5)	1.6	0.8 (-0.6-5.2)
73+ years	277	1144.8	1	1.04	1.0	(0.0-5.4)	0.9	0.0 (-0.9-4.0)
Histological Classification								
Intestinal Metaplasia	446	2581.4	5	2.01	2.5	(0.8-12.6)	1.9	1.2 (-0.2-3.7)
Barrett's NOS	159	978.7	0	0.77	-	-	-	-
Dysplasia								
No dysplasia	493	2958.1	4	2.24	1.8	(0.5-4.6)	1.4	0.6 (-0.4-2.7)
Low-grade dysplasia	92	487.6	0	0.42	-	-	-	-
High-grade dysplasia	8	44.5	1	0.04	24.8	(0.6-137.9)	22.5	21.6 (-0.4-124.3)
Unspecified dysplasia	12	69.9	0	0.07	-	-	-	-
Follow-up duration								
0-<1 years	605	578.9	1	0.43	2.3	(0.1-12.8)	1.7	1.0 (-0.7-8.9)
1-4 years	549	1745.2	3	1.34	2.2	(0.5-6.5)	1.7	1.0 (-0.4-4.3)
5-9 years	318	1006.6	.	0.80	.	..	-	-
10+ years	110	229.4	1	0.20	5.1	(0.1-28.5)	4.4	3.5 (-0.7-23.4)

^a Barrett's cases with esophageal and gastric cancer before diagnosis or within half year after diagnosis of Barrett's esophagus are excluded (leaving 605 cases at risk and 3560.0 person-years at risk); ^b O/E ratio = Observed/expected ratio; 95% CI = 95% Confidence Intervals; NOS = Not Otherwise Specified; BE= Barrett's esophagus; AER= Absolute excess risk; ^c Calculated as ((Observed-Expected)*1000)/person-years; ^d The first six months of follow-up were excluded. T=0 starts therefore at six months.

Table 4.3 Cancer incidence in Barrett's esophagus cohort^a for selected sites (n=561). Netherlands Cohort Study, 1986-2003.

ICD-10	Primary site ^b	Observed (n)	Expected (n)	O/E-ratio ^c	95% CI
C00-C43, C45-C96	All Malignant, skin cancer excluded	92	63.3	1.5	(1.2-1.8)
C00-C14, C16-C43, C45-C96	All Malignant, skin, stomach & esophagus cancer excluded	74	59.5	1.2	(1.0-1.6)
C00-C14, C30-C32, C65	Head & Neck	3	2.0	1.5	(0.3-4.4)
C15-C26	Digestive Organs	34	16.6	2.0	(1.4-2.9)
C15	Esophagus	13	1.2	11.0	(5.9-18.8)
C16	Stomach	5	2.5	2.0	(0.6-4.6)
C17	Small Intestine	2	0.2	11.8	(1.4-42.5)
C18-C20	Colon & Rectum	9	10.0	0.9	(0.4-1.7)
C25	Pancreas	5	1.6	3.1	(1.0-7.2)
C30-C39	Respiratory & Intrathoracic Organs	15	11.6	1.3	(0.7-2.1)
C34	Lung & Bronchus	14	10.7	1.3	(0.7-2.2)
C40-C41	Bone & Joint	0	0.1	-	-
C43	Skin, Melanoma	2	1.1	1.9	(0.2-6.8)
C45-C49	Mesothelial & Soft Tissue	1	0.8	1.2	(0.0-6.8)
C50	Breast	7	6.2	1.1	(0.5-2.3)
C51-C58	Female Genital Organs	2	2.6	0.8	(0.1-2.8)
C60-C63	Male Genital Organs	15	10.0	1.5	(0.8-2.5)
C61	Prostate	15	9.9	1.5	(0.9-2.5)
C64-C68	Urinary Tract	6	4.8	1.2	(0.5-2.7)
C69-C72	Eye, Meninges, Central Nervous System	4	1.5	2.7	(0.7-7.0)
C73-C75	Endocrine Glands	1	0.7	1.5	(0.0-8.4)
C76-C80	Primary Unknown or Ill Defined	0	0.2	-	-
C81-C96	Blood, Bone Marrow & Lymphatic organs	4	4.4	0.9	(0.3-2.4)

^a No prevalent cancer (skin cancer excluded) at diagnosis of Barrett's esophagus (561 cases at risk and 3382.8 person-years); ^b All main categories are presented. Subsites are presented if >5 observed cases or if O/E-ratio statistically significant decreased or increased; ^c O/E-ratio = Observed/expected ratio; 95% CI = 95% Confidence Interval.

^d Statistically significantly increased.

Table 4.4 Total and cause-specific mortality in Barrett's esophagus cohort according to cause of death (n=626)^a. Netherlands Cohort Study, 1986-2005.

ICD-10 codes	Cause of Death	Observed (n)	Expected (n)	O/E-ratio ^b	95% CI
A00-Y89	All Causes ^c	225	213.2	1.1	(0.9-1.2)
A00-B99	Infectious & Parasitic diseases	1	2.3	0.4	(0.0-2.4)
D50-D89	Blood(-forming) & Immune system	0	0.6	-	-
E00-E90	Endocrine, Nutritional & Metabolic	7	6.6	1.1	(0.4-2.2)
F00-F90	Mental & Behavioral disorders	4	7.1	0.6	(0.2-1.4)
G00-H95	Central & Peripheral nervous system	9	4.8	1.9	(0.9-3.6)
I00-I99	Circulatory system	84	80.0	1.1	(0.8-1.3)
I21-I22	Myocardial infarction	23	21.9	1.1	(0.7-1.6)
I20, I23-I25	Other Ischemic heart disease	13	8.8	1.5	(0.8-2.5)
I30-I33, I39-I52	Other Heart disease	17	18.1	0.9	(0.6-1.5)
I60-I69	Cerebrovascular disease	17	18.8	0.9	(0.5-1.4)
I00-I15, I26-I28, I34-I38, I70-I99	Other Circulatory diseases	31	12.4	1.1	(0.6-1.9)
J00-J99	Respiratory system	22	23.8	0.9	(0.6-1.4)
J10-J11	Influenza	0	0.3	-	-
J12-J18	Pneumonia	6	8.1	0.7	(0.3-1.6)
J40-J47	COPD & Asthma	14	13.1	1.1	(0.6-1.8)
J00-J06, J20-J39, J60-J99	Other Respiratory organs	2	2.3	0.9	(0.1-3.1)
K00-K93	Digestive system	7	7.5	0.9	(0.4-1.9)
L00-L99	Skin & Subcutaneous tissue	0	0.5	-	-
M00-M99	Musculoskeletal & Connective tissue	0	1.3	-	-
N00-N99	Urogenital system	4	4.2	1.0	(0.3-2.4)
P00-P96	Perinatal period	0	0.0	-	-
Q00-Q99	Congenital malformations	0	0.1	-	-
R00-R99	Symptoms & signs, NOS	6	8.1	0.7	(0.3-1.6)
V01-Y89	External causes	5	4.1	1.2	(0.4-2.9)
V01-V99	Vehicle (transport) accidents	2	0.7	2.9	(0.4-10.6)
W00-X59	Other Accidents	3	2.5	1.2	(0.3-3.5)
X60-X84	Suicide (intentional self-harm)	0	0.7	-	-
X85-Y09	Kill, Murder & Homicide	0	0.0	-	-
Y10-Y89	Other External events	0	0.2	-	-

^a 626 persons at risk and 4490.5 person-years; ^b O/E ratio = Observed/expected ratio; 95% CI = 95% Confidence Interval; COPD = Chronic Obstructive Pulmonary Disease; NOS= Not Otherwise Specified; ^c Including mortality because of cancer.

DISCUSSION

In this large population-based cohort of cases with Barrett's esophagus, only the O/E-ratios of esophageal cancer were statistically significantly increased. Presence of intestinal metaplasia and unspecified or high-grade dysplasia were associated with the highest risks of esophageal cancer, although numbers were small. Cancer incidence without esophageal and gastric cancer incidence was slightly increased, but not statistically significantly. Incidence of small intestinal and pancreatic cancer was increased, but was based on small numbers and not statistically significant after excluding the first half year of follow-up. Cause-specific and overall mortality were not increased.

Barrett's esophagus is considered to be a precursor lesion of esophageal adenocarcinoma.²⁰ In agreement with this assumption, we observed a strongly increased risk of esophageal cancer during follow-up. The incidence rate in the cohort was 3.7 per 1000 person-years after excluding the first half year of follow-up. The pooled estimate in a recent meta-analysis was estimated to be 5.3 per 1000 person-years.⁴ However, when the pooled analysis was restricted to studies with a large study size (>500 person-years), without possible selection bias (>70% follow-up) and a well-defined definition of Barrett's esophagus, the incidence was considerably lower and very close to the results in the current study: 3.9 per 1000 person-years.⁴ In a recent follow-up study, an even lower incidence of esophageal cancer was observed: only 1.6 per 1000 person-years.³ The largest follow-up study, using the same Pathology database as the current study, found an incidence rate of 4.3 per 1000 person-years.⁵ The relatively low incidence in these studies confirms the opinion that the incidence of esophageal cancer in Barrett's esophagus has been overestimated in the literature because of publication bias.²⁰

In the current study, the incidence rate of esophageal cancer in males was much higher than that in females, which is in agreement with the meta-analysis.⁴ Cases with unspecified or high-grade dysplasia at baseline had a strongly increased risk of esophageal cancer, although the O/E-ratios were based on one case in each category. The increased risk is in accordance with the literature.^{21,22} The increased risk in cases with unspecified dysplasia is possibly explained by classification problems. In the summaries of the pathology reports of these cases, dysplasia was sometimes reported without specification as low- or high-grade, and we classified these cases as unspecified dysplasia. The elevated risk may therefore be explained by this misclassification. In contrast to findings from a recent large study,⁵ the O/E-ratios were highest during the first five years of follow-up. It is conceivable, that the incidence after five years of follow-up was underestimated in our study, because the number of BE cases and cancer events is small, especially regarding cases with a longer follow-up.

The incidence rate of gastric cancer was also slightly increased. In a recent cohort study from Cook et al the O/E-ratio of gastric cancer was 2.0 (7 cases of gastric cancer observed and 3.43 expected),³ which is comparable with the result in the current study

(O/E-ratio: 1.8). Because most gastric cancers in the current study were located in the gastric cardia, the increased risk might be caused by misclassification of esophageal cancer as gastric cardia cancer. However, the Netherlands Cancer Registry has good access to clinical files and its data have been shown to be of good quality,²³ making misclassification less likely.

Incidence rates of small intestinal and pancreatic cancer were also increased, but not statistically significantly after exclusion of the cases in the first six months after BE diagnosis. It is therefore conceivable that preclinical complaints of these cancers warranted endoscopy, in which the Barrett's esophagus was discovered. The number of observed cases in our study is also limited. However, in patients with Multiple Endocrine Neoplasia type 1 (MEN1) and the Zollinger Ellison Syndrome (ZES) the risks of both duodenal and endocrine pancreatic malignancies and of Barrett's esophagus were increased.^{24,25} In many patients with ZES, excess gastrin is produced by a gastrinoma in e.g. the duodenum. Gastrin increases gastric acid production and might therefore increase the risk of Barrett's esophagus. This is in agreement with the observation in the current study that one of the detected duodenal cancers was reported to be a neuroendocrine carcinoma (carcinoid). This finding should be replicated in other longitudinal studies, before it can be concluded whether a causal association is likely.

Barrett's esophagus has also been associated with an increased incidence of colorectal cancer.²⁶ However, results since this first publication in 1985 have been inconsistent.^{3,7,27} The possible association between Barrett's esophagus and colorectal cancer has been attributed to common environmental exposures such as bile acids, vegetables and fruit intake, and shared genetic aberrations.²⁶ A recent study, using data from the Netherlands Pathology Registry, showed a slightly increased risk of colorectal cancer, but incidence was especially increased in the first year of follow-up.⁶ The authors concluded that the greater part of the excess risk is most likely explained by diagnostic bias. We did not observe an increased risk at all, making it likely that there is no or only a small increased risk of colorectal cancer in patients with Barrett's esophagus.

The O/E-ratio for total mortality was slightly increased, but not statistically significantly. None of the specific causes of death (excluding cancer mortality) was increased. Some, but not all, recent studies observed moderately increased O/E-ratios for mortality. In a British cohort study with 502 patients, an increased mortality was observed (O/E ratio 1.21; 95% CI: 1.06-1.37).³ Several other studies also reported increased mortality rates.⁸⁻¹¹ Risks in these studies remained elevated after exclusion of deaths that were related to esophageal cancer. Only one study did not observe an increased risk.¹² In this study, data were used from the Northern Irish Registry of Barrett's esophagus, and the O/E ratio was 0.96 (95% CI, 0.84-1.07). The current study and the Irish study are the only studies that are population-based and not hospital-based. A possible explanation for the observed discrepancies might therefore be selection bias. The hospital-based cohorts were assembled at hospitals and may

include patients with more complicated disease or with more co-morbidities and were therefore at increased risk to die. Lack of power is not a likely explanation, as the current study has 225 deaths, which is comparable with the other published studies.^{3,11,12}

This study suffered from some limitations. Case definitions of Barrett's esophagus differ between the United States (USA) and the United Kingdom (UK). In the USA, the presence of goblet cells (indicating intestinal metaplasia) is required for the diagnosis of BE,²⁸ while in the UK any type of metaplasia is sufficient for the diagnosis of BE.²⁹ Both the USA and UK definitions may have been used in the Netherlands. The use of definitions may have changed over time and different pathologists may have used different definitions. Cases were excluded from the analysis if the pathological summary only reported non-intestinal metaplasia (gastric or junctional metaplasia). In the pathology reports of approximately 25% of the cases the type of Barrett's esophagus was not specified. Some misclassification may therefore have occurred and this may have attenuated the risks slightly. A second limitation is that we only had access to summaries of the pathology reports as recorded in the pathology registry PALGA. However, we were able to compare the conclusions of 60 reports from 29 patients with the full reports from one hospital. In this substudy, the agreement on patient level was 29/29 (100%) with respect to the eligibility of the cases, 26/29 (90%) with respect to histology and 27/29 (93%) with respect to dysplasia.

Our study also has several strengths. The study is population-based and has a long follow-up. Follow-up for vital status was 100% complete and we were able to retrieve all causes of death from the death certificates. Follow-up for cancer is also presumed to be >95% complete.³⁰

In conclusion, in this study we observed that patients with Barrett's esophagus had an increased risk of esophageal cancer and possibly also for stomach, small intestinal and pancreatic cancer. The incidence of colorectal cancer was not increased. Neither overall nor cause-specific mortality were increased.

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5

Trends in incidence of esophageal and stomach cancer subtypes in Europe

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ABSTRACT

Objective

Time trend studies in the USA have shown that the incidences of adenocarcinomas of the esophagus and gastric cardia have risen strongly since the 1970s, whereas the incidence of squamous cell carcinomas of the esophagus has declined. Previously, we found that the incidence of these adenocarcinomas also rose in some European countries until the early 1990s. The main goal of this study was to investigate more recent trends in the incidence of esophageal and stomach cancer subtypes in European countries.

Methods

Eurocim cancer incidence data of 23 cancer registries from 13 European countries were used to investigate the incidence trends in esophageal and stomach cancer subtypes in the 1983-1997 period. We calculated estimated annual percent changes (EAPCs) in European age- standardized incidence rates and 95% confidence intervals.

Results

The incidence of adenocarcinomas of the esophagus and gastric cardia rose in most, but not all, registration areas (EAPCs were usually 1 to 7%), the strongest in the UK and Ireland. Esophageal squamous cell carcinoma incidence rose mostly in Northern European and Slovakian men (EAPCs: 1 to 5%) and in women (EAPCs: 1 to 8%), but declined mostly in Southern and Western European men (EAPCs: -1 to -5%).

Conclusions

Our results are partly in line with earlier findings on adenocarcinomas of the esophagus and gastric cardia. There was, however, substantial heterogeneity in trends of subtypes of these cancers within Europe. There may be different risk factors for these cancers, and the prevalence of these risk factors may differ among countries.

INTRODUCTION

These days, esophageal cancer and stomach cancer are often being considered to comprise more than two types of cancer (e.g. in epidemiology, pathology). Esophageal cancer is usually divided into histological subtypes, the two main types being adenocarcinoma and squamous cell carcinoma. Subtypes of stomach cancer are usually based on topography (i.e. the location of the tumor in the stomach), distinguishing cardia tumors from tumors in other parts of the stomach. The incidences of both esophageal and stomach cancers are relatively high¹ and the 5-year survival rates are very low: 12% for esophageal cancer and 24% for stomach cancer in Europe.² Survival rates of gastric cardia adenocarcinoma are worse than those of other stomach cancers.^{3,4}

Time trend studies in the United States have shown that the incidences of adenocarcinomas of the esophagus and gastric cardia (AEGC) have risen strongly from the 1970s, whereas the incidence of squamous cell carcinomas of the esophagus has declined.^{5,6} In an earlier publication, we investigated whether these changes also took place in Europe. We showed that this rise in the incidence of AEGC did indeed occur in Denmark, part of Italy, Slovakia, England and Wales and Scotland, in the 1980s and early 1990s.⁷

Such time trends should always be interpreted cautiously, as an observed trend may reflect changes in diagnostics, in classification of tumors or in the quality of cancer registries. The quality and level of detail of data on esophageal and stomach cancer incidences have increased over time.⁸⁻¹⁰ More esophageal tumors are being histologically verified and the exact topography of stomach tumors is more often being registered.⁸⁻¹⁰ In contrast, observed trends can of course also indicate a true change in the occurrence of the disease, which may follow a change in the prevalence of one or more risk factors. There are indications that the subtypes of esophageal and stomach cancer have different risk factors.^{e.g.11}

Two studies have investigated these trends in Europe in more recent years,^{12,13} but one did not include any Southern or Eastern European countries¹² and the other studied only esophageal cancers.¹³

The main goals of the present study were therefore: (a) to study trends in the incidence of histological subtypes of esophageal cancer and topographical subtypes of stomach cancer, and (b) to include data from all European regions. This allowed a comparison between cancers and between regions as well. We used Eurocim¹⁴ data and we presented the results in a conveniently arranged way.

METHODS

Eurocim database

Cancer registries that are members of the European Network of Cancer Registries are asked to regularly submit incidence, mortality and population data to a central databank (Eurocim¹⁴) held at the International Agency for Research on Cancer (IARC). We obtained permission from the steering committee of the European Network of Cancer Registries to use the Eurocim database, which contains incidence data from 118 population-based cancer registries in 25 countries. Available registration years range from 1953 to 1998. In addition, the National Cancer Registry of Ireland has extracted data for the area covered by the Southern Tumors Registry for the period 1983-1997, because these data were not available in the present version of the Eurocim database. We wanted to include this region, because it was also included in our earlier study.⁷

Selection of cancer registries

Figure 5.1 gives an overview of the registry selection, and Table 5.1 lists all registries included. A cancer registry had to be situated in Europe and had to have registration data available in Eurocim of esophageal and stomach cancer, for a period of at least 15 years. To ensure that all Europe was covered in this study, at least one registry in every country was selected. If several registries were available in one country, we selected those with best data quality. Data quality of cancer registries is defined by three indicators: (a) the percentage of histologically or microscopically verified cases (%HV), (b) the percentage of cases derived from death certificates only (%DCO) and (c) the ratio of mortality to incidence (M/I ratio). We looked at the indicator values from Cancer Incidence in Five Continents (CI-5) volumes IV-VIII.^{8-10,15,16} Not all quality indices were available for all registries; mostly the %DCO was missing and sometimes the M/I ratio.

From 27 of 29 selected registries, we obtained permission to use their Eurocim data. The registries were asked some questions regarding registration practice, as this could be of importance when interpreting the results. Four registries were excluded from the study, because they did not have data that were detailed enough in terms of esophageal cancer histology or stomach cancer topography. Data of 23 cancer registries from 13 countries were available for analysis and all included the 1983-1997 period. When compared with the earlier analyses,⁷ this study covered more countries and five more years.

Figure 5.1 Selection of cancer registries and reasons for exclusion.

Selection of tumors

We included all primary tumors of the esophagus and stomach (International Classification of Diseases for Oncology version 2 (ICD-O-2)¹⁷ codes C15 and C16, respectively). Esophageal cancers were divided in three histological subgroups: (a) squamous cell carcinomas (ESCC), (b) adenocarcinomas and (c) other esophageal cancers. Stomach cancers were divided into three topographical subgroups: (a) cardia, (b) other specified sites (OSS) and (c) not otherwise specified (NOS) (Appendix).

We then combined AEGC to form one group (Appendix). Although it is subject of discussion whether or not these diseases are the same,^{4,18,19} it was necessary to combine them, because some cancer registries used to classify adenocarcinomas of the lower esophagus as cardia tumors, as was also advised by the International Classification of Diseases version 9 (ICD-9). In the analysis, we distinguished stomach tumors OSS from NOS, so that we could examine whether trends observed in cardia

tumors could be partly explained by increasing registry quality or specification of tumor sublocalisation.

Table 5.1 Characteristics of selected cancer registries, by region.

	Population size in 1997 (x 1 million)	Esophageal cancer cases (n/year) ^a		Stomach cancer cases (n/year) ^a	
		male	female	male	female
Northern Europe					
Denmark	5.3	167	75	470	310
Estonia	1.5	47	11	290	245
Iceland	0.3	7	5	44	23
Ireland, Southern ^b	0.6	23	18	46	26
Sweden	8.8 ^c	225	96	1034	664
UK England, East Anglia ^d	2.2	126	82	266	140
UK England, Merseyside and Cheshire ^e	2.4	152	117	358	249
UK England, South Thames Region	6.9	306	235	868	599
UK England, Yorkshire ^f	3.7	196	138	477	304
UK Scotland	5.1	333	274	684	509
Central & Eastern Europe					
Slovakia	5.4	138	18	903	555
Southern Europe					
Italy, Parma	0.4	14	4	146	103
Italy, Lombardy (Varese)	0.8	37	7	168	130
Slovenia	2.0	77	13	294	202
Spain, Navarra	0.5	23	4	102	60
Spain, Catalonia, Tarragona	0.6	21	2	64	37
Western Europe					
France, Bas-Rhin	1.0	88	8	82	55
France, Calvados	0.6	86	8	55	37
France, Côte d'Or	0.5	37	4	43	25
France, Doubs	0.5 ^c	34	4	39	23
the Netherlands, Eindhoven	1.0	16	7	99	59
Switzerland, Geneva	0.4	18	5	34	27
Switzerland, St Gallen Appenzell	0.5	17	3	44	32

^a Average number of cases in the 1983-1996 period. This period is available in Eurocim for all selected registries; ^b Data were obtained from the National Cancer Registry of Ireland. Southern Ireland represents the area formerly covered by the Southern Tumour Registry; ^c Population size of 1998 (Sweden), population size of 1996 (France, Doubs). These years were the latest available in Eurocim; ^d The registry's current name is Eastern Cancer Registration & Information Service (ECRIC); ^e This registry is now part of the North West Cancer Intelligence Service (NWCIS); ^f This registry is now part of the Northern and Yorkshire Cancer Registry and Information Service (NYCRIS).

Statistical analyses

Data analyses were performed using Eurocim version 4.0 and Stata 9.2 (StataCorp, College Station, Texas, USA). We calculated age-standardized incidence rates (ESR) according to the European Standard Population.¹⁴ A graph of these rates against time was plotted for each registry and sex, showing trends in the five tumor groups mentioned above. These graphs were inspected to see whether the trends were linear and whether a change in trend (a joinpoint) seemed to be present. In case a joinpoint

seemed to be present, we used the Joinpoint regression program (Version 3.0, Statistical Research and Applications Branch, National Cancer Institute, Bethesda, MD, USA) to test whether the trend change was statistically significant.²⁰ The minimal number of joinpoints was set to zero, and the maximum to three; which are the default values of the program.

Estimated annual percentage changes (EAPC) and corresponding 95% confidence intervals (95% CI) were calculated for each registry, sex and cancer subtype, using the following formulae: $\ln(\text{rate}) = a \times \text{period} + b$ and $\text{EAPC} = 100 (e^a - 1)$, where a is regression coefficient and b is intercept. The EAPCs were calculated for the same 15-year period (1983-1997) for all registries to make these comparable with one another. Data of different cancer registries within one country were not pooled before analysis for two reasons: (a) this would make differences within in a country indiscernible and (b) regional registries included do not always cover the whole country (e.g. France, UK). In addition, French regional cancer registries are not representative for the national population.²¹ The EAPCs were visualized in forest plots, to facilitate comparisons of the trends in Europe. Results are presented by regions of Europe, according to the United Nations classification.²² For each region, a summary estimate is presented, which was obtained by a fixed effects model using the method of Mantel and Haenszel. The I-squared measure is shown to quantify the heterogeneity within the regions.²³

RESULTS

Descriptives

Table 5.1 lists some characteristics of the 23 cancer registries included. The population size covered by the cancer registries ranged from 0.3 to 8.8 million. The average numbers of cancer cases per year are listed in Table 5.1. For esophageal cancer, these ranges were 7-333 cases per year in men and 2-274 cases per year in women and for stomach cancer, these ranges were 34-1034 and 23-664 cases per year in men and women, respectively. ESRs, averaged over three-year periods, were higher among men than among women (for details, see Tables 5.2A and 5.2B). Figure 5.2 shows two examples of ESR graphs.

The male-to-female ratios of ESRs varied greatly between countries and between the cancer subtypes. These were highest for ESCC: about six to nine, averaged for all registries. Very high ratios were seen in France, Spain, Switzerland (St Gallen Appenzell) and Slovakia. In contrast, the ratios were just above one in Great Britain and Southern Ireland. Much less variation was observed in the male-to-female ratios of AEGC, with an average ratio of approximately five. Lower average ratios (approximately two) were found for stomach cancers OSS and NOS.

Table 5.2A Annual incidence rates according to the European Standard Population (ESR^a) of esophageal and stomach cancer subtypes, men.

	Esophageal squamous cell carcinoma		Adenocarcinomas of esophagus and gastric cardia		Stomach other specified sites		Stomach not otherwise specified	
	1982-1984 ^b	1995-1997	1982-1984	1995-1997	1982-1984	1995-1997	1982-1984	1995-1997
Northern Europe								
Denmark	3.0	3.4	6.6	7.7	5.0	2.3	9.7	5.3
Estonia	3.4	5.8	5.8	3.6	30.3	29.0	20.4	13.7
Iceland	5.2	5.2	7.0	7.7	10.2	6.7	27.4	12.8
Ireland, Southern ^c	3.9	5.4	5.4	10.9	3.1	8.9	15.3	3.0
Sweden	3.5	2.6	3.3	4.4	0	0	18.9	9.8
UK England, East Anglia ^d	2.7	3.3	7.0	14.9	5.7	6.0	14.6	7.8
UK England, Merseyside and Cheshire ^e	3.7	3.6	8.7	14.6	18.0	6.0	11.5	10.3
UK England, South Thames Region	2.7	2.9	7.0	10.1	5.7	3.0	15.0	10.1
UK England, Yorkshire ^f	3.1	3.4	8.3	13.0	7.6	4.7	15.1	11.2
UK Scotland	4.9	5.7	8.7	15.1	6.8	4.3	18.7	12.3
Central & Eastern Europe								
Slovakia	3.5	8.1	3.2	4.0	23.5	22.7	17.5	5.4
Southern Europe								
Italy, Parma	3.0	4.4	3.5	5.3	28.8	22.9	32.2	11.6
Italy, Lombardy (Varese)	6.8	6.3	4.1	5.0	32.3	24.9	18.1	3.7
Slovenia	7.0	5.9	4.8	4.6	9.7	13.1	25.2	17.3
Spain, Navarra	8.5	7.7	3.0	5.1	16.0	23.1	22.4	5.4
Spain, Catalonia, Tarragona	5.3	5.4	2.4	2.3	3.1	5.4	19.2	13.7
Western Europe								
France, Bas-Rhin	25.1	14.9	5.0	6.3	4.1	4.7	16.6	9.5
France, Calvados	32.8	18.3	6.0	7.0	20.5	13.6	1.0	2.0
France, Côte d'Or	15.7	8.5	4.7	7.0	18.9	9.6	0.4	0
France, Doubs	14.5	12.4	7.6	3.4	16.9	7.8	1.8	4.5
the Netherlands, Eindhoven	3.1	2.8	7.0	8.7	19.4	15.7	4.6	1.2
Switzerland, Geneva	8.6	7.5	5.2	5.8	12.3	8.7	1.5	0.7
Switzerland, St Gallen Appenzell	9.6	3.5	6.4	7.0	14.8	9.7	0.5	2.4

^a ESR, incidence rate according to the European Standard Population. Number of cases per 100 000 person-years; ^b Average incidences rates over 3-year periods were calculated to create more stable rates. When only 2 years of the 3-year period were available, a 2-year average was calculated; ^c Data were obtained from National Cancer Registry of Ireland; ^d The registry's current name is Eastern Cancer Registration & Information Service (ECRIC); ^e This registry is now part of the North West Cancer Intelligence Service (NWCIS); ^f This registry is now part of the Northern and Yorkshire Cancer Registry and Information Service (NYCRIS).

Table 5.2B Annual incidence rates according to the European Standard Population (ESR^a) of esophageal and stomach cancer subtypes, women

	Esophageal squamous cell carcinoma		Adenocarcinomas of esophagus and gastric cardia		Stomach other specified sites		Stomach not otherwise specified	
	1982-1984 ^b	1995-1997	1982-1984	1995-1997	1982-1984	1995-1997	1982-1984	1995-1997
Northern Europe								
Denmark	1.1	1.5	1.6	1.4	2.4	1.7	5.7	2.8
Estonia	0.5	0.7	1.5	1.1	17.6	13.9	8.8	5.9
Iceland	3.3	2.4	1.8	1.6	7.1	3.2	11.1	5.0
Ireland, Southern ^c	4.5	3.3	0.9	2.9	1.0	5.0	7.5	2.0
Sweden	1.1	0.9	0.7	1.0	0	0	10.3	5.7
UK England, East Anglia ^d	2.4	3.2	1.5	2.9	2.1	2.1	6.6	2.9
UK England, Merseyside and Cheshire ^e	3.1	3.5	2.4	3.7	7.7	2.8	5.5	5.3
UK England, South Thames Region	2.3	2.4	1.4	2.2	2.5	1.3	6.3	4.1
UK England, Yorkshire ^f	2.6	2.6	1.6	3.3	3.4	2.4	6.9	5.0
UK Scotland	3.5	4.5	2.6	4.2	3.6	2.3	10.1	6.5
Central & Eastern Europe								
Slovakia	0.2	0.4	0.8	0.9	10.5	10.1	9.1	2.5
Southern Europe								
Italy, Parma	0.5	1.2	0.7	0.8	14.4	13.8	14.4	6.7
Italy, Lombardy (Varese)	0.7	0.9	0.9	1.6	15.7	13.8	9.3	2.9
Slovenia	0.8	0.6	1.2	1.3	3.5	6.1	12.4	7.8
Spain, Navarra	0.7	0.3	0.7	0.8	6.8	9.9	8.0	1.7
Spain, Catalonia, Tarragona	0.4	0.4	0.5	0.4	1.9	2.3	9.5	5.6
Western Europe								
France, Bas-Rhin	0.9	1.2	0.8	0.8	2.4	2.6	6.5	3.4
France, Calvados	1.7	2.3	1.6	1.4	8.4	6.2	0.5	1.0
France, Côte d'Or	0.9	1.5	1.1	0.5	7.7	4.5	0.4	0.2
France, Doubs	1.4	2.1	1.4	1.2	8.9	4.1	0.7	2.2
the Netherlands, Eindhoven	1.1	1.8	2.3	1.9	9.8	7.2	1.4	0.5
Switzerland, Geneva	1.8	2.7	1.1	1.2	5.0	5.4	1.6	0.4
Switzerland, St Gallen Appenzell	0.3	0.9	1.1	1.2	12.3	4.8	0.4	1.1

^a ESR, incidence rate according to the European Standard Population. Number of cases per 100 000 person-years; ^b Average incidences rates over 3-year periods were calculated to create more stable rates. When only 2 years of the 3-year period were available, a 2-year average was calculated; ^c Data were obtained from National Cancer Registry of Ireland; ^d The registry's current name is Eastern Cancer Registration & Information Service (ECRIS); ^e This registry is now part of the North West Cancer Intelligence Service (NWCIS); ^f This registry is now part of the Northern and Yorkshire Cancer Registry and Information Service (NYCRIS).

Figure 5.2 Trends in incidence rates (according to the European Standard Population, ESR) of esophageal and stomach cancer subtypes for men and women in Slovenia (A) and UK Scotland (B), 1983-1997

Estimated annual percentage changes

Figures 5.3A to 5.3H show EAPCs and 95% CIs for ESCC (Figures 5.3A and 5.3B), AEGC (5.3C and 5.3D), stomach tumors OSS (5.3E and 5.3F) and stomach tumors NOS (5.3G and 5.3H), for men and women separately, during the 1983-1997 period. The EAPCs were calculated for all groups, but the incidence rates for women were often very low, which resulted in wide confidence intervals for these EAPCs.

ESCC incidence rose slightly; approximately 1 to 2% per year in most Northern European countries and Slovakia in men and women, but none of the EAPCs was

statistically significant. In Southern and Western Europe the incidence declined approximately 1 to 5% per year in men, and mostly rose in women. The latter EAPCs were based on less than 10 cases per year, which is reflected by the wide 95% CIs. AEGC incidence rose in men and women in most (but not all) European regions covered by the cancer registries. The increases were strongest in Northern Europe (1 to 7% per year), specifically in Great Britain and Southern Ireland (approximately 3 to 7%). Smaller increases (1 to 3% per year) were seen in the other European regions. Individual cancer registration areas where the incidence of AEGC did not rise were: Estonia, Iceland, Slovenia, Spain Navarra (women), Spain Tarragona, France Doubs, Netherlands Eindhoven (women) and Switzerland Geneva (men). The above trends were not always statistically significant; for details see Figures 5.3A-H.

In Northern European men and Southern European women, AEGC was more common than ESCC. This difference had become larger during the 1983-1997 period. In Northern European women, AEGC also became more frequent relative to ESCC, and by the end of the 1990s both types had become equally common. A similar tendency was seen in men from Southern and Western Europe, although AEGC had not, or not yet, outnumbered ESCC. In contrast, the ratio of ESCC to AEGC increased in Western European women. The incidence of other esophageal tumors was considerably low in most countries, when compared with AEGC and ESCC (data not shown).

Stomach tumors OSS showed a declining incidence in nearly all registration areas, in men as well as in women. The greatest declines were seen in Western and Northern Europe, but the picture was less clear for Southern Europe and Slovakia. A remarkable exception was Southern Ireland, where a strong and statistically significant increase in this type of cancer was found (EAPCs: 16% in men and 18% in women).

Incidence of stomach tumors NOS declined (approximately 2 to 10% per year) in both sexes in practically all registration areas in Northern, Central & Eastern, and Southern Europe, and the decline was often statistically significant. In Western Europe, the incidence of these tumors also declined in some areas but, in contrast, increased in some parts of France and Switzerland. In the latter regions, however, this relative increase represents a small absolute increase, as the percentage of stomach tumors NOS was already low in the early 1980s, because of a good topographic specification.

Joinpoint analyses

Results of the Joinpoint analyses showed that when a change in trend seemed present, it was mostly also statistically significant (data not shown). The majority of trend changes were seen in stomach tumors NOS and some in stomach tumors OSS. A strong decline in the incidence of stomach tumors NOS was mostly followed by a weaker decline. We did not observe any trend changes in the incidence of AEGC. In only one registration area (France Bas-Rhin) a trend change was observed in ESCC incidence in men: it rose until 1984 and declined thereafter.

A

B

C

D

E

F

G

H

Figure 5.3 Estimated annual percent changes (EAPCs) and 95% confidence intervals of standardized incidence rates (according to the European Standard Population, ESR) of esophageal and stomach cancer subtypes in European countries, 1983-1997.

A summary estimate of the EAPCs is depicted for each European region, which was obtained by a fixed effects model using the method of Mantel and Haenszel. The I-squared measure quantifies the heterogeneity within the regions.

DISCUSSION

Summary of results

This study showed that the incidence of adenocarcinomas of esophagus and gastric cardia (AEGC) rose for both sexes in many, but not all, European countries during the 1983-1997 period. The magnitude of this increase differed among countries. Incidence rates of ESCC rose in some European regions, but declined in others, and there was a difference between men and women. In nearly all regions, the ratio of AEGC to ESCC incidence was increasing.

Interpretation of trends in incidence

When interpreting results of studies on trends in incidence rates, one always has to be cautious. A careful look should be taken at possible explanations, other than a true increase of the disease. The growing use of endoscopies may have caused more tumors of the esophagus to be histologically verified, but we do not expect this growing use of endoscopies to have caused much overdiagnosis of esophageal cancers. The attention for esophageal and stomach cancer increased and might have influenced pathologists, and the histological description of the tumors became more detailed. This may have led to improved detection of these tumors. In some countries the incidence of not specified esophageal tumors indeed decreased, but mainly before the period studied and the incidence of these tumors is generally low.

A better specification and registration of the topography of tumors could in part explain the decreasing trend in stomach cancers NOS, which goes together with the increase of cardia tumors. However, the decline of stomach cancers NOS is often much stronger than the increase in cardia tumors. Furthermore, one would then expect stomach cancers OSS to increase as well, but in most countries these showed a decreasing incidence instead. In Southern Ireland, the trends (rising AEGC and stomach cancers OSS and declining stomach cancers NOS) may in part have been caused by a better topography registration. However, we do not know the quality indices of the mid 1990s to support this.

In this study we did not separate adenocarcinomas of the esophagus from tumors of the gastric cardia. Some studies have made this separation and have found different trends in incidence for these tumors.^{5,12,18,24} There are studies indicating that these two cancers have a different etiology,^{e.g.25} but other studies point to similar risk factors.^{e.g.11} It is difficult to distinguish tumors of the lower esophagus from those of the gastric cardia. This applies in the clinic and in cancer registration, because registration differs among registries and over time. Therefore, combining the two tumors was the best solution.

Comparison with other studies

In the literature, rising incidences in AEGC were found in the USA, Australia and New Zealand,^{5,12,18,24} but no such trend was found in Hong Kong and Taiwan.^{26,27} The results of our study show that beside heterogeneity in trends between continents, heterogeneity can also be found within Europe, which is consistent with results from our earlier study.⁷

Eurocim database only contains data up to 1997, but it is a valuable source of data for trend studies in which sublocalisation and histological subgroups are needed, and should therefore be updated regularly. A comparison with more recent data from individual countries is limited, because, to our knowledge there are only very few studies available. In Sweden, the trends in the incidences of ESCC, esophageal adenocarcinoma, and gastric cardia adenocarcinoma, continued in the same direction until 2004.²⁸ In the Netherlands, trends were described for AEGC until 2003, showing a further increase in incidence in men, and in women as well.²⁹ In France, the incidences of esophageal cancer and stomach cancer were estimated until 2000 and were expected to decrease, except for stomach cancer in women, which was thought to rise.³⁰ These figures, however, are estimated for the whole country, whereas individual regions may have different trends, as we also found in our study. In Italy, the incidence of stomach cancer was estimated until 2010 and was thought to further decrease.³¹

Possible explanations for the trends observed

If the observed trends represent real changes in incidence, what then underlies these changes? Ecological studies compare trends in the prevalence of possible risk factors with the observed trends in cancer incidence. Trend data of these factors however, are mostly not available until the 1980s, whereas earlier data are probably more relevant, because one expects a certain latency time before changes in risk factors have their effect on cancer incidence. Therefore, the following must be interpreted cautiously. Results from the MONICA study showed that the prevalence of obesity increased between the mid 1980s and late 1990s,³² most strongly in the UK. This may explain part of the trends in incidence of AEGC as overweight and obesity are strong risk factors for these tumors.¹¹ The rising prevalence described in that study can affect AEGC incidence later on. The decreasing prevalence of *Helicobacter pylori* infection in developed countries³³ could also be important, because this infection is related to increased risk of noncardia stomach cancer, but to decreased risk of AEGC.^{34,35} Smoking and alcohol consumption are differently related to the subtypes of esophageal and stomach cancer^{36,37} and Bosetti et al. concluded that these factors might explain trends in incidence.¹³ In addition, other factors (e.g. diet and nutrition) could explain the observed trends.

Suggestions for the future

Eurocim database is a valuable source of information for all kinds of studies, and should therefore be updated regularly. If the Eurocim database would be updated, data from more countries could be used because they will then have data available for a longer period. This is especially important with respect to Eastern Europe, which was not well represented in this study. Thus there is a need for better standards in timeliness and comparability in cancer registry datasets.

Etiological epidemiological research can identify environmental risk factors for esophageal and stomach cancer and its subtypes and should be applied in primary prevention, as survival is still very low.

APPENDIX

Classification of tumors of the esophagus and stomach

Description of tumor	Topography codes ICD-O-2 ¹⁷	Morphology codes ICD-O-2 ¹⁷	Eurocim entities ¹⁴
Esophagus, squamous cell carcinoma (ESCC)	C15.0-C15.9	8050-8082	134, 141, 148
Esophagus, adenocarcinoma ^a	C15.0-C15.9	8140-8473, 8480-8490, 8500-8550	135-136, 142-143, 149-150
Esophagus, other subtypes	C15.0-C15.9	All other morphologies	137-140, 144-147, 151-154
Stomach, cardia ^a	C16.0	All morphologies	155-161
Stomach, other specified sites (OSS)	C16.1-C16.8	All morphologies	162-175
Stomach, not otherwise specified (NOS)	C16.9	All morphologies	176-182

^a These tumors were combined in analyses into adenocarcinomas of the esophagus and gastric cardia (AEGC).

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6

Alcohol consumption, cigarette smoking and risk of subtypes of esophageal and gastric cancer: a prospective cohort study

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ABSTRACT

Objective

Alcohol consumption and cigarette smoking may be differentially associated with esophageal squamous cell carcinoma (ESCC), esophageal adenocarcinoma (EAC), gastric cardia adenocarcinoma (GCA), and gastric non-cardia adenocarcinoma (GNCA). However, because this was based on retrospective studies, these hypotheses were examined in a prospective cohort.

Methods

The prospective Netherlands Cohort Study consists of 120 852 participants who completed a baseline questionnaire on diet and other cancer risk factors in 1986. After 16.3 years of follow-up, 107 ESCC, 145 EAC, 164 GCA, and 491 GNCA cases were available for analysis using Cox proportional hazards models and the case-cohort approach.

Results

The multivariable adjusted incidence rate ratio (RR) for ESCC was 4.61 (95% confidence interval (CI) 2.24-9.50) for ≥ 30 g ethanol/day compared with abstainers (p trend < 0.001), while no associations with alcohol were found for EAC, GCA or GNCA. Compared with never smokers, current smokers had RRs varying from 1.60 for GCA to 2.63 for ESCC, and were statistically significant or borderline statistically significant. Frequency, duration, and pack-years of smoking were independently associated with risk of all four cancers. A positive interaction was found between alcohol consumption and smoking status regarding ESCC risk. The RR for current smokers who consumed > 15 g/day of ethanol was 8.05 (95% CI 3.89-16.60; p interaction = 0.65), when compared with never smokers who consumed < 5 g/day of ethanol.

Conclusions

This prospective study found alcohol consumption to be associated with increased risk of only ESCC. Cigarette smoking was associated with risk of all four cancers.

INTRODUCTION

In the past, esophageal and gastric cancer have been regarded as two disease entities, but nowadays it is becoming clearer that probably each represents several diseases. The two main subtypes of esophageal cancer are squamous cell carcinoma (ESCC) and adenocarcinoma (EAC). Gastric cancer can be subdivided into gastric cardia adenocarcinomas (GCA) and gastric non-cardia adenocarcinomas (GNCA).

The incidence rates of EAC and GCA have risen greatly in the USA and Europe during the past decades.^{1,2} For ESCC, conversely, rates declined or remained stable, and rates of GNCA declined greatly.^{1,2} These differences in trends indicate that these cancer subtypes may have a different etiology and thus differential associations with lifestyle factors such as alcohol consumption and cigarette smoking.

On this topic, mainly case-control studies have been performed, but only few have compared subtypes of esophageal and gastric cancer.³⁻⁶ This study design is sensitive for misclassification,⁷ which might be of importance in studies investigating smoking and alcohol consumption, because of participants' awareness of the established health risks of these exposures.⁸ Prospective cohort studies are less susceptible to this bias. However, only two cohort studies^{9,10} have reported on alcohol consumption and risk of ESCC, EAC, GCA and GNCA, and three cohort studies have reported on cigarette smoking.⁹⁻¹¹ These studies often lacked sufficient information on confounders.^{9,11} We therefore investigated this association within a large-scale prospective cohort: the Netherlands Cohort Study on diet and cancer (NLCS).¹²

We hypothesized that: (1) alcohol consumption and cigarette smoking are strongly positively associated with ESCC risk, with multiplicative interaction; (2) alcohol consumption is not associated with EAC, GCA, and GNCA; and (3) cigarette smoking is positively associated with EAC, GCA, and GNCA, but less strongly than with ESCC.

METHODS

Study design and participants

This study was conducted within the NLCS, which started in September 1986 with the enrolment of Dutch men (n=58 279) and women (n=62 573) women aged 55-70 years.¹²

For reasons of efficiency, the case-cohort approach was used for data processing and analysis.¹³ Cases were derived from the entire cohort, and the number of person-years at risk for the entire cohort was estimated from a subcohort of 5000, who were randomly sampled from the total cohort at baseline. Person-years at risk were calculated from the start of the study until esophageal or gastric cancer diagnosis, death, emigration, loss to follow-up or end of follow-up, whichever occurred first.

Follow-up for cancer incidence was performed by record linkage to the Netherlands Cancer Registry (NCR) and the nationwide network and registry of histopathology and cytopathology in the Netherlands (PALGA).¹⁴ The completeness of cancer follow-up is $\geq 96\%$,¹⁵ and follow-up of the subcohort was nearly 100% complete (only one male subcohort member was lost to follow-up) after 16.3 years (from 17 September 1986 until 31 December 2002). The following numbers of incident, microscopically confirmed, primary carcinomas were identified: 130 ESCC¹⁶ (ICD-O-3 C15), 181 EAC¹⁶ (C15), 206 GCA (C16.0) and 594 GNCA (C16.1-C16.9) (Figure 6.1). Excluded were non-microscopically confirmed cancers, which formed 1% of both esophageal and gastric cancers. The group of GNCA included cancers with a lesion with overlapping subsites of the stomach (C16.8, $n=160$) and some gastric not otherwise specified cancers (C16.9, $n=75$), raising the possibility that some cardia cancers might be included in the non-cardia category. However, as we found risk estimates to be similar in separate analyses of gastric cancers of specified sites (C16.1-C16.5) and other gastric cancers (C16.6-C16.9) (data not shown), we combined the groups in the analysis.

We excluded subcohort members who reported having prevalent cancer other than skin cancer at baseline. Also excluded were cases and subcohort members with incomplete or inconsistent dietary data¹⁷ or missing data on exposure or confounding variables. Figure 6.1 shows that 3962 subcohort members and 107 ESCC, 145 EAC, 164 GCA, and 491 GNCA cases were available for analysis.

Exposure information

All cohort members completed a self-administered questionnaire at baseline. This questionnaire included a 150-item food frequency questionnaire (FFQ), with questions on alcohol consumption, and questions on other cancer risk factors, such as smoking habits, level of education, body mass index (BMI), physical activity, family history of cancer and use of medication.

We asked about the habitual consumption of alcohol during the year preceding the start of the study, and this was measured by six items: (1) beer, (2) red wine, (3) white wine, (4) sherry and other fortified wines, (5) liquor types containing on average 16% alcohol and (6) (Dutch) gin, brandy and whisky. Questions were asked about the frequency of consumption and the number of glasses consumed on each drinking occasion. For analysis, we combined (2), (3) and (4) into “wine”, and (5) and (6) into “liquor”. Mean daily alcohol consumption was calculated using the Dutch food-composition table.¹⁸ Based on a pilot study, a glass of beer, wine and liquor was assumed to contain 200, 105 and 45 ml of the beverage, respectively (unpublished findings). For “beer” and “other alcoholic beverages”, participants could indicate whether five years ago, they drank (1) more than, (2) equal amounts of or (3) less than today. The fourth answering option was (4) “I never use this”. Using these questions,

we selected those participants with stable alcohol consumption to perform a sensitivity analysis to investigate the robustness of our results.

Figure 6.1 Flow diagram of subcohort members and cases on whom the analyses were based. *PALGA: nationwide network and registry of histopathology and cytopathology in The Netherlands.

Regarding cigarette smoking, questions were asked about the following: whether the subject was a smoker at baseline, age at which they started smoking, age at smoking cessation, the number of cigarettes smoked daily and the number of smoking years (excluding stopping periods). Based on these questions, the following variables were constructed: smoking status (never, former, current), current smoking (yes/no), frequency (number of cigarettes/day), duration (number of years), pack-years of cigarette smoking (number), and time since cessation (years).

The FFQ has been validated against a 9-day diet record, and the Spearman correlation coefficient between the alcohol intake assessed by the questionnaire and that estimated by the diet record was 0.89 for all subjects and 0.85 for users of alcoholic beverages.¹⁷ The reproducibility of the FFQ was established and the test-retest correlation was 0.90 for alcohol intake, and this correlation declined only 0.01-0.02 per year.¹⁹ This indicates that the single FFQ measurement was able to rank

subjects according to alcohol intake and this ability dropped only slightly over time. The single FFQ measurement that is used in our cohort study can characterize dietary habits for a period of at least 5 years.¹⁹

Questionnaire data were key-entered and processed in a standardized manner, blinded with respect to case/subcohort status in order to minimize observer bias in coding and data interpretation.

Data analysis

The confounders considered were:²⁰⁻²² age (years), sex, level of education (primary, lower vocational, secondary and medium vocational, and university and higher vocational), BMI (kg/m^2), non-occupational physical activity (<30, 30 to <60, 60 to <90, ≥ 90 min/day), energy intake (kJ), tea consumption (cups/day), intakes of fruit, vegetables, legumes, fish, red meat and meat products (all g/day), family history of esophageal or gastric cancer (yes/no in first-degree relatives), reported long-term (>0.5 years) use of non-steroidal anti-inflammatory drugs (NSAIDs) or aspirin (ATC codes M01A, N02B) (yes/no), and reported long-term use (yes/no) of lower esophageal sphincter (LES)-relaxing medication.²³⁻²⁵ Alcohol consumption and cigarette smoking were mutually adjusted in the statistical models. A variable was considered a confounder if including it in the model changed the rate ratio (RR) for any of the cancer types by >5%. For all four cancers, the same confounders were used in analyses.

Incidence RRs and corresponding 95% CIs for alcohol consumption and cigarette smoking were estimated in age- and sex-adjusted and multivariable adjusted case-cohort analyses using Cox proportional hazards models.²⁶ Analyses were done using Stata 9.2 statistical software package (StataCorp, College Station, Texas, USA). Standard errors were estimated using the robust Huber-White sandwich estimator to account for additional variance introduced by sampling from the cohort. This method is equivalent to the variance-covariance estimator by Barlow.²⁷ The proportional hazards assumption was tested using the scaled Schoenfeld residuals.²⁸ If the assumption was violated for a confounder, a time-varying covariate was added to the model. In case the assumption was violated for the exposure variable, it was checked whether the time-varying covariate for this variable was statistically significant in the model. Tests for dose-response trends were assessed by fitting ordinal exposure variables as continuous terms. Two-sided p values are reported throughout the article.

To evaluate whether substances in alcoholic beverages, other than ethanol, have an effect on the risk of ESCC, EAC, GCA, or GNCA, we also analyzed beer, wine, and liquor consumption, adjusted for ethanol intake.

In smoking analyses, the different aspects of smoking were mutually adjusted for, to investigate which aspect is most important in terms of esophageal or gastric cancer risk.

Interaction

To evaluate possible interaction between alcohol and cigarette smoking status, we estimated RRs of all four cancers for combinations of these exposures. The p value for interaction was assessed by including a cross-product term in the model. In the interaction analyses it was necessary to change the alcohol categories to include subjects with low alcohol consumption in the reference category, besides abstainers, because of too low numbers of cases per stratum.

RESULTS

Descriptives

Table 6.1 presents the characteristics of the subcohort members and cases, including all variables that were considered potential confounders. Esophageal and gastric cancer cases were more often men, especially EAC and GCA cases. Alcohol consumption was higher in cases, especially ESCC cases, than in subcohort members, while the proportion of alcohol abstainers was higher in the subcohort. About 60% of subcohort members and cases were stable alcohol consumers. Cases were exposed more to cigarette smoke in terms of smoking status, frequency, duration, and pack-years compared with the subcohort.

Cases also differed from subcohort members with respect to some other characteristics: EAC and GCA cases had a higher BMI than subcohort members. All cases consumed less fruit and slightly less vegetables than the subcohort, but cases more often reported a family history of esophageal or gastric cancer and use of LES-relaxing medication (Table 6.1).

The proportional hazards assumption was violated only in a few analyses (see footnotes in Table 6.2 and Supplemental Table 6.1 available online).

Alcohol consumption

A daily alcohol consumption of ≥ 30 g, when compared with abstaining, was associated with a significantly increased risk of ESCC (multivariable adjusted RR=4.61, 95% CI 2.24 to 9.50, p trend<0.001) (Table 6.2). Women were at somewhat higher risk than men, and the interaction with sex was statistically significant in continuous analyses ($p=0.04$), but not in categorical analyses ($p=0.68$). No association was observed between alcohol consumption and EAC (RR for ≥ 30 g/day=1.04, 95% CI 0.54 to 2.02), GCA (RR=0.90, 95% CI 0.50 to 1.64) or GNCA (RR=1.00, 95% CI 0.68 to 1.47).

Multivariable RRs for alcohol consumption and ESCC were slightly attenuated when compared with age- and sex-adjusted RRs (see Supplemental Table 6.1). For EAC, GCA, and GNCA, age- and sex-adjusted RRs were very similar to multivariable adjusted RRs.

Table 6.1 Characteristics of cases and subcohort members in the Netherlands Cohort Study on diet and cancer (NLCS), 1986-2002.

Exposure variables and potential confounders	Subcohort	Cases			
	(n=3962) ^a	ESCC (n=107) ^a	EAC (n=145) ^a	GCA (n=164) ^a	GNCA (n=491) ^a
age at baseline (years)	61.3 (4.2)	62.5 (4.2)	61.6 (4.1)	61.5 (4.1)	62.5 (4.1)
sex					
men (n (%))	1944 (49.1)	59 (55.1)	114 (78.6)	140 (85.4)	331 (67.4)
women (n (%))	2018 (50.9)	48 (44.9)	31 (21.4)	24 (14.6)	160 (32.6)
abstainer from alcohol (n (%))	943 (23.8)	16 (15.0)	25 (17.2)	29 (17.7)	107 (21.8)
alcohol consumers:					
ethanol intake (g/day)	13.6 (15.1)	27.6 (27.9)	17.3 (18.3)	16.6 (15.5)	15.9 (17.2)
beer intake (glasses/day)	0.3 (0.8)	0.8 (1.8)	0.5 (1.1)	0.5 (1.0)	0.5 (1.2)
wine intake (glasses/day)	0.5 (0.8)	0.6 (1.1)	0.4 (0.7)	0.4 (0.7)	0.4 (0.8)
liquor intake (glasses/day)	0.5 (0.8)	1.1 (1.7)	0.7 (1.0)	0.7 (0.9)	0.6 (0.9)
cigarette smoking status					
never smoker (n (%))	1460 (36.9)	22 (20.6)	28 (19.3)	28 (17.1)	113 (23.0)
former smoker (n (%))	1425 (36.0)	31 (29.0)	70 (48.3)	80 (48.8)	201 (40.9)
current smoker (n (%))	1077 (27.2)	54 (50.5)	47 (32.4)	56 (34.2)	177 (36.1)
ever cigarette smokers:					
frequency of cigarette smoking (n/day)	15.3 (10.3)	18.3 (10.3)	20.1 (12.6)	17.5 (11.1)	16.5 (10.4)
duration of cigarette smoking (years)	31.6 (12.2)	36.9 (10.5)	34.0 (11.0)	34.9 (11.4)	34.7 (11.8)
pack-years of cigarette smoking (n)	22.7 (17.7)	30.3 (19.0)	30.9 (20.4)	27.2 (18.0)	26.3 (19.2)
level of education					
primary (n (%))	1104 (27.9)	34 (31.8)	37 (25.5)	45 (27.4)	185 (37.7)
lower vocational (n (%))	868 (21.9)	22 (20.6)	37 (25.5)	42 (25.6)	117 (23.8)
secondary and medium vocational (n (%))	1420 (35.8)	38 (35.5)	49 (33.8)	45 (27.4)	142 (28.9)
university and higher vocational (n (%))	570 (14.4)	13 (12.2)	22 (15.2)	32 (19.5)	47 (9.6)
body mass index (kg/m ²)	25.0 (3.1)	24.2 (3.6)	26.3 (3.4)	25.7 (3.0)	24.9 (3.2)
non-occupational physical activity (min/day)	73 (60)	68 (65)	74 (58)	86 (78)	80 (71)
energy intake (kJ/day)					
men	9090 (2130)	8703 (2033)	9054 (2025)	8834 (2160)	9166 (2112)
women	7063 (1651)	7406 (1806)	6981 (1807)	7445 (1510)	6995 (1510)
tea consumption (cups/day)	2.8 (2.0)	2.7 (2.4)	2.6 (2.3)	2.3 (1.8)	2.8 (1.9)
fruit consumption (g/day)	177 (120)	130 (108)	169 (135)	154 (116)	164 (125)
vegetable consumption (g/day)	194 (83)	189 (76)	187 (83)	188 (84)	188 (81)
legumes consumption (g/day)	8 (12)	7 (10)	10 (12)	9 (13)	8 (11)
fish consumption (g/day)	13 (15)	14 (13)	15 (19)	15 (20)	14 (19)
red meat consumption (g/day)	87 (40)	93 (41)	90 (37)	92 (45)	91 (41)
meat products consumption (g/day)	13 (15)	14 (14)	14 (15)	15 (16)	15 (16)
family history of esophageal or gastric cancer (n (%))	296 (7.5)	10 (9.4)	18 (12.4)	14 (8.5)	63 (12.8)
reported long-term use of NSAIDs (n (%))	262 (6.6)	7 (6.5)	11 (7.6)	9 (5.5)	23 (4.7)
reported long-term use of LES relaxing medication (n (%))	564 (14.2)	17 (15.9)	28 (19.3)	25 (15.2)	77 (15.7)

^a The number of subcohort members or cases used in age- and sex-adjusted analyses and multivariable analyses of alcohol consumption and cigarette smoking. Values are given as mean (SD); for categorical variable n (%) is presented. EAC, esophageal adenocarcinoma; ESCC, esophageal squamous cell carcinoma; GCA, gastric cardia adenocarcinoma; GNCA, gastric non-cardia adenocarcinoma; LES, lower esophageal sphincter; NSAIDs, non-steroidal anti-inflammatory drugs.

When the analyses were restricted to stable alcohol consumers, the association with ESCC became somewhat stronger. The results for EAC, GCA, and GNCA barely changed (Table 6.2).

Results regarding alcoholic beverages are shown in table 2. After adjustment for total alcohol intake, beer consumption was associated with an increased risk of ESCC (p trend=0.23) and GNCA (p trend = 0.06). Wine consumption was inversely associated with risk of ESCC (RR per 1 glass/day increment=0.67, 95% CI 0.50 to 0.90), but was not associated with EAC, GCA or GNCA. Consumption of liquor was not significantly associated with risks of ESCC, EAC, and GCA, and with a reduced risk of GNCA.

Cigarette smoking

Current and former cigarette smoking were associated with an increased risk of all four cancer types, when compared with never smokers (Table 6.3). The strongest associations were found for ESCC and GNCA.

Frequency of cigarette smoking was found to be associated with increased risks of all four cancers, independent of other smoking aspects. The association was statistically significant for ESCC and EAC. The RRs for the gastric cancers were lower than for the esophageal cancers (Table 6.3). Duration of cigarette smoking was independently associated with increased risks of ESCC, GCA, and GNCA, but the RR was only statistically significant for GNCA. Pack-years of smoking were associated with increased risks (mostly statistically significant) of all four cancers. The highest RRs were found in those who smoked ≥ 40 pack-years.

We also looked into the effect of cigarette smoking cessation on the risk of esophageal and gastric cancers. The risks of all four cancer types declined for smokers who had stopped since <10, 10 to <20 or ≥ 20 years (p trend <0.05 for all four cancers), when compared with current smokers. However, when compared with never smokers, the risks were still elevated for ESCC, EAC, and GNCA. For ESCC, EAC, and GCA, the RRs of time since smoking cessation were attenuated when we adjusted for smoking duration (data not shown).

Multivariable RRs for cigarette smoking were attenuated when compared with age- and sex-adjusted RRs (Supplemental Table 6.2). This difference could largely be explained by the mutual adjustment of cigarette smoking aspects. For ESCC, confounding by alcohol consumption also caused attenuation of the associations.

Table 6.2 Association (multivariable^a adjusted incidence RRs) between alcohol consumption and risk of esophageal and gastric cancer subtypes; Netherlands Cohort Study on diet and cancer (NLCS) 1986-2002.

Subcohort		Esophageal squamous cell carcinoma			Esophageal adenocarcinoma			Gastric cardia adenocarcinoma			Gastric non-cardia adenocarcinoma		
categorical median	person time at risk (years)	no. of cases	RR	(95% CI)	no. of cases	RR	(95% CI)	no. of cases	RR	(95% CI)	no. of cases	RR	(95% CI)
Alcohol consumption (g ethanol/day)													
abstainer	13336	16	1	(reference)	25	1	(reference)	29	1	(reference)	107	1	(reference)
>0<5	16740	15	0.85	(0.42-1.73)	38	1.17	(0.69-1.98)	30	0.75	(0.44-1.30)	121	0.92	(0.69-1.23)
5<15	12714	23	1.65	(0.85-3.17)	30	0.91	(0.51-1.60)	47	1.11	(0.68-1.82)	126	1.07	(0.80-1.43)
15<30	8979	24	2.11	(1.08-4.14)	31	1.01	(0.56-1.82)	36	0.92	(0.54-1.57)	78	0.77	(0.55-1.08)
>=30	5037	29	4.61	(2.24-9.50)	21	1.04	(0.54-2.02)	22	0.90	(0.50-1.64)	59	1.00	(0.68-1.47)
continuous, 10 grams ethanol/day increments				p trend ^b <0.001			p trend 0.93			p trend 0.99			p trend 0.68
overall	56806	107	1.32	(1.19-1.45)	145	1.01	(0.90-1.14)	164	0.98	(0.88-1.08)	491	1.02	(0.95-1.09)
men	26679	59	1.28	(1.15-1.43)	114	0.99	(0.88-1.12)	140	0.98	(0.89-1.09)	331	1.03	(0.95-1.11)
women	30128	48	1.62	(1.31-2.00)	31	1.23	(0.93-1.64)	24	0.84	(0.41-1.75)	160	0.96	(0.78-1.18)
Alcohol consumption (g ethanol/day)				p interaction ^c 0.04			p interaction 0.41			p interaction 0.65			p interaction 0.47
Stable users ^d													
abstainer	10763	13	1	(reference)	21	1	(reference)	23	1	(reference) ^e	88	1	(reference)
>0<5	9780	8	0.74	(0.30-1.80)	19	0.91	(0.48-1.73)	12	0.46	(0.22-0.97)	75	0.91	(0.65-1.28)
5<15	7831	13	1.59	(0.72-3.49)	19	0.88	(0.46-1.68)	22	0.78	(0.41-1.47)	75	0.98	(0.69-1.39)
15<30	4932	14	2.44	(1.06-5.60)	18	1.07	(0.53-2.16)	19	0.83	(0.41-1.69)	39	0.65	(0.42-1.01)
>=30	2735	15	5.34	(2.16-13.18)	15	1.43	(0.66-3.11)	10	0.76	(0.34-1.73)	33	0.98	(0.60-1.61)
continuous, 10 g ethanol/day increments				p trend <0.001			p trend 0.28			p trend 0.79			p trend 0.52
overall	36041	63	1.42	(1.24-1.63)	92	1.08	(0.95-1.24)	86	0.99	(0.85-1.14)	310	0.99	(0.89-1.09)

Subcohort		Esophageal squamous cell carcinoma			Esophageal adenocarcinoma			Gastric cardia adenocarcinoma			Gastric non-cardia adenocarcinoma			
		person time at risk (years)	no. of cases	RR (95% CI)	no. of cases	RR (95% CI)	no. of cases	RR (95% CI)	no. of cases	RR (95% CI)	no. of cases			
Alcoholic beverages (glasses/day) ^f														
Beer														
no beer	0	38324	60	1	(reference)	79	1	(reference)	86	1	(reference ^g)	294	1	(reference)
>0-1	0.2	14606	28	0.98	(0.57-1.67)	48	0.84	(0.55-1.28)	59	0.86	(0.59-1.25)	140	0.90	(0.70-1.15)
>1-2	1.4	2543	8	1.36	(0.59-3.16)	10	0.84	(0.41-1.71)	12	0.86	(0.44-1.68)	30	1.02	(0.65-1.60)
>2	3.7	1333	11	1.62	(0.64-4.09)	8	1.07	(0.44-2.62)	7	0.90	(0.39-2.07)	27	1.58	(0.95-2.63)
				ptrend 0.23			ptrend 0.80			ptrend 0.87			ptrend 0.06	
continuous, 1 glass/day increments		56806	107	1.10	(0.92-1.32)	145	0.98	(0.82- 1.17)	164	1.03	(0.86- 1.24)	491	1.15	(1.03-1.29)
Wine														
no wine	0	26060	50	1	(reference ^g)	74	1	(reference)	84	1	(reference)	268	1	(reference)
>0-1	0.2	23467	42	0.93	(0.60-1.44)	55	0.97	(0.64-1.47)	64	1.01	(0.69-1.49)	169	0.88	(0.70-1.10)
>1-2	1.4	4806	8	0.51	(0.22-1.17)	11	0.92	(0.45-1.87)	9	0.69	(0.31-1.54)	36	0.91	(0.59-1.39)
>2	2.7	2473	7	0.30	(0.07-1.23)	5	0.79	(0.28-2.20)	7	1.04	(0.40-2.70)	18	0.88	(0.48-1.63)
				ptrend 0.05			ptrend 0.64			ptrend 0.70			ptrend 0.73	
continuous, 1 glass/day increments		56806	107	0.67	(0.50-0.90)	145	0.89	(0.67- 1.19)	164	0.87	(0.62-1.21)	491	0.93	(0.77-1.13)
Liquor														
no liquor	0	29359	41	1	(reference)	60	1	(reference)	60	1	(reference)	230	1	(reference)
>0-1	0.2	20833	31	1.13	(0.69-1.84)	53	0.96	(0.65-1.41)	69	1.14	(0.79-1.65)	183	0.93	(0.74-1.15)
>1-2	1.9	4714	21	1.82	(0.97-3.41)	20	1.11	(0.61-2.03)	29	1.43	(0.81-2.53)	59	0.84	(0.58-1.21)
>2	2.8	1900	14	1.55	(0.64-3.78)	12	1.53	(0.68-3.48)	6	0.72	(0.24-2.18)	19	0.58	(0.33-1.03)
				ptrend 0.11			ptrend 0.36			ptrend 0.68			ptrend 0.12	
continuous, 1 glass/day increments		56806	107	1.21	(0.92-1.60)	145	1.12	(0.87-1.43)	164	1.07	(0.79-1.45) ^e	491	0.90	(0.76-1.07)

^a Adjusted for age, sex, cigarette smoking (current smoking status (yes/no), frequency, and duration), body mass index, level of education, energy intake, consumption of fruits, vegetables, fish; ^b Tests for dose-response trends were assessed by fitting ordinal variables as continuous terms in the Cox proportional hazard model; ^c p value for interaction between sex and alcohol consumption, based on cross product term in the Cox proportional hazard model; ^d Subjects who had not changed their alcohol consumption habits in the five years before baseline; ^e Proportional hazards assumption was violated for the exposure variable, but there was no statistically significant interaction with time; ^f Additionally adjusted for ethanol intake; ^g Proportional hazards assumption was violated for the exposure variable, and there was a statistically significant interaction with time. CI, confidence interval; RR, rate ratio.

Table 6.3 Association (multivariable adjusted^a incidence rate ratios) between cigarette smoking and risk of esophageal and gastric cancer subtypes; Netherlands Cohort Study on diet and cancer (NLCS) 1986-2002.

Subcohort		Esophageal squamous cell carcinoma		Esophageal adenocarcinoma		Gastric cardia adenocarcinoma		Gastric non-cardia adenocarcinoma	
category	person time at risk (years)	no. of cases	RR (95% CI)	no. of cases	RR (95% CI)	no. of cases	RR (95% CI)	no. of cases	RR (95% CI)
Smoking status									
never smokers	21958	22	1 (reference)	28	1 (reference)	28	1 (reference)	113	1 (reference)
former smokers	20271	31	1.38 (0.72-2.63)	70	1.47 (0.92-2.35)	80	1.40 (0.86-2.27)	201	1.47 (1.11-1.96)
current smokers	14577	54	2.63 (1.47-4.69)	47	1.67 (1.01-2.77)	56	1.60 (0.97-2.66)	177	1.86 (1.40-2.48)
			p trend ^b <0.001		p trend 0.05		p trend 0.07		p trend <0.001
Smoking status, additional adjustment for smoking frequency and duration									
never smokers	21958	22	1 (reference)	28	1 (reference)	28	1 (reference)	113	1 (reference)
former smokers	20271	31	0.68 (0.27-1.74)	70	0.81 (0.37-1.77)	80	0.80 (0.37-1.76)	201	1.09 (0.70-1.70)
current smokers	14577	54	1.07 (0.38-3.04)	47	0.87 (0.31-2.40)	56	0.74 (0.27-2.04)	177	1.26 (0.71-2.25)
			p trend 0.27		p trend 0.98		p trend 0.63		p trend 0.34
Frequency of cigarette smoking (n/day)									
no cigarette smoker	0	22	1 (reference)	28	1 (reference)	28	1 (reference)	113	1 (reference)
>0 - <20	10	44	0.79 (0.32-1.97)	50	0.98 (0.46-2.06)	74	0.84 (0.39-1.81)	222	1.16 (0.76-1.77)
≥20	20	41	1.15 (0.44-2.99)	67	1.77 (0.80-3.88)	62	0.92 (0.40-2.14)	156	1.31 (0.81-2.11)
			p trend 0.24		p trend 0.01		p trend 0.82		p trend 0.22
continuous, 10 cigarettes/day increments	56806	107	1.18 (1.00-1.40)	145	1.26 (1.08-1.46)	164	1.04 (0.89-1.22)	491	1.09 (0.98-1.20)
Duration of cigarette smoking (years)									
no cigarette smoker	0	22	1 (reference)	28	1 (reference)	28	1 (reference)	113	1 (reference)
>0 - <20	12	4	0.48 (0.15-1.57)	13	0.78 (0.37-1.66)	16	1.04 (0.51-2.11)	52	1.34 (0.90-1.98)
20 - <40	30	33	1.26 (0.60-2.67)	61	1.11 (0.64-1.92)	59	1.28 (0.72-2.27)	153	1.27 (0.91-1.77)
≥40	43	48	1.72 (0.73-4.03)	43	0.89 (0.45-1.73)	61	1.62 (0.82-3.20)	173	1.51 (1.00-2.29)
			p trend 0.08		p trend 0.88		p trend 0.14		p trend 0.10
continuous, 10 years increments	56806	107	1.11 (0.93-1.33)	145	1.04 (0.90-1.20)	164	1.14 (0.98-1.33)	491	1.10 (1.00-1.20)

	Subcohort	Esophageal squamous cell carcinoma			Esophageal adenocarcinoma			Gastric cardia adenocarcinoma			Gastric non-cardia adenocarcinoma		
		person time at risk (years)	no. of cases	RR (95% CI)	no. of cases	RR (95% CI)	no. of cases	RR (95% CI)	no. of cases	RR (95% CI)	no. of cases	RR (95% CI)	
Pack-years of cigarette smoking													
no cigarette smoker	0	21958	22	1 (reference)	28	1 (reference)	28	1 (reference)	113	1 (reference)			
>0 - <20	9	18505	25	1.07 (0.53-2.17)	38	1.15 (0.68-1.94)	52	1.23 (0.73-2.07)	154	1.32 (0.98-1.78)			
20 - <40	28	11247	39	2.14 (1.02-4.46)	42	1.72 (0.97-3.05)	55	1.67 (0.96-2.90)	153	1.88 (1.34-2.63)			
≥40	48	5096	21	2.22 (1.01-4.86)	37	2.93 (1.59-5.40)	29	1.74 (0.93-3.25)	71	1.68 (1.12-2.52)			
				p trend 0.01		p trend <0.001		p trend 0.04		p trend 0.004			
Continuous, 10 pack-years increments		56806	107	1.14 (1.03-1.25)	145	1.16 (1.07-1.26)	164	1.07 (0.99-1.16)	491	1.08 (1.02-1.15)			
Smoking cessation													
never smokers		21958	22	1 (reference)	28	1 (reference)	28	1 (reference)	113	1 (reference)			
stopped ≥ 20 years	25	6280	10	1.46 (0.63-3.42)	19	1.32 (0.70-2.47)	18	1.00 (0.53-1.91)	49	1.13 (0.77-1.67)			
stopped 10 - <20 years	14	6953	10	1.28 (0.58-2.84)	23	1.42 (0.80-2.51)	28	1.43 (0.81-2.52)	66	1.41 (0.98-2.02)			
stopped >0 - <10 years	5	6984	11	1.42 (0.62-3.23)	28	1.66 (0.95-2.91)	34	1.72 (0.97-3.05)	85	1.81 (1.30-2.52)			
current smokers	0	14577	54	2.63 (1.47-4.69)	47	1.68 (1.01-2.78)	56	1.61 (0.97-2.66)	177	1.86 (1.39-2.47)			
				p trend 0.001		p trend 0.03		p trend 0.02		p trend <0.001			

^a all analyses were adjusted for age, sex, alcohol consumption, body mass index, level of education, energy intake, consumption of fruits, vegetables, fish. Analyses of smoking frequency, duration and pack-years of smoking were adjusted for current smoking status (yes/no). Additionally, smoking frequency and duration were mutually adjusted for in analyses; ^b Tests for dose-response trends were assessed by fitting ordinal variables as continuous terms in the Cox proportional hazard model. CI, confidence interval; RR, rate ratio.

Interaction

Table 6.4 shows RRs for several exposure combinations of alcohol consumption and cigarette smoking, using never smokers and low alcohol consumers as a reference. A graphical presentation of these results can be found in Figure 6.2A-D.

The RR of ESCC was 8.05 (95% CI 3.89 to 16.60) in the category of current smokers who consumed >15 g/day of ethanol. This RR is compatible with multiplicative interaction, but was not statistically significant (p interaction=0.65). We found no evidence for interaction between alcohol consumption and cigarette smoking on risk of EAC, GCA, or GNCA.

Figure 6.2 Combined exposure to alcohol and cigarette smoking and risk of esophageal and gastric cancer subtypes; Netherlands Cohort Study on diet and cancer, 1986-2002. (A) Esophageal squamous cell carcinoma, (B) Esophageal adenocarcinoma, (C) Gastric cardia adenocarcinoma, (D) Gastric non-cardia adenocarcinoma. Multivariable RRs: adjusted for age, sex, body mass index, level of education, energy intake, consumption of fruits, vegetables, fish.

Table 6.4 Combinations of alcohol consumption and cigarette smoking and risk (multivariable adjusted^a incidence RRs) of esophageal and gastric cancer subtypes; Netherlands Cohort Study on diet and cancer (NLCS) 1986-2002.

Cigarette smoking status	Esophageal squamous cell carcinoma			Esophageal adenocarcinoma			Gastric cardia adenocarcinoma			Gastric non-cardia adenocarcinoma		
	Alcohol consumption (g ethanol/ day)			Alcohol consumption (g ethanol/ day)			Alcohol consumption (g ethanol/ day)			Alcohol consumption (g ethanol/ day)		
	0-5	>5-15	>15	0-5	>5-15	>15	0-5	>5-15	>15	0-5	>5-15	>15
never smoker												
cases/person time at risk												
(years)	14/16351	3/3750	5/1857	18/16351	5/3750	5/1857	18/16351	6/3750	4/1857	81/16351	18/3750	14/1857
RR	1 (ref)	1.01	3.74	1 (ref)	0.92	1.76	1 (ref)	0.92	1.15	1 (ref)	0.89	1.40
95% CI		0.28-3.59	1.25-11.20		0.32-2.67	0.61-5.06		0.35-2.41	0.37-3.54		0.52-1.52	0.76-2.58
former smoker												
cases/person time at risk												
(years)	8/7830	8/5600	15/6842	28/7830	14/5600	28/6842	23/7830	31/5600	26/6842	73/7830	63/5600	65/6842
RR	1.33	2.04	3.16	1.79	1.24	1.72	1.15	2.08	1.18	1.41	1.78	1.40
95% CI	0.53-3.35	0.81-5.15	1.36-7.31	0.93-3.44	0.58-2.64	0.88-3.39	0.57-2.31	1.10-3.91	0.61-2.32	0.98-2.02	1.21-2.62	0.96-2.04
current smoker												
cases/person time at risk												
(years)	9/5896	12/3364	33/5317	17/5896	11/3364	19/5317	18/5896	10/3364	28/5317	74/5896	45/3364	58/5317
RR	1.70	4.48	8.05	1.87	1.87	1.81	1.51	1.27	1.89	2.04	2.18	1.62
95% CI	0.72-4.05	1.97-10.20	3.89-16.60	0.92-3.82	0.83-4.21	0.87-3.79	0.75-3.01	0.56-2.90	0.97-3.68	1.43-2.91	1.45-3.27	1.09-2.41
	p interaction ^b 0.65			p interaction 0.79			p interaction 0.29			p interaction 0.42		

^a Adjusted for age, sex, body mass index, level of education, energy intake, consumption of fruits, vegetables, fish; ^b p value for interaction between cigarette smoking status and alcohol consumption was assessed by including a cross-product term in the Cox proportional hazard model. CI, confidence interval; ref, reference category; RR, rate ratio.

DISCUSSION

Main findings

In this study, which is one of the first prospective cohort studies on the topic, we found the following results. Alcohol consumption was related to a strongly increased risk of ESCC, whereas it was unrelated to EAC, GCA, or GNCA. Cigarette smoking status, frequency, duration, and pack-years were associated with increased risks of all four cancer types. A multiplicative interaction was found between alcohol consumption and cigarette smoking on the risk of ESCC, but no such interaction existed for EAC, GCA, or GNCA.

Strengths and limitations

A strength of our study includes the prospective character, which makes selection and information bias unlikely. Moreover, the division we made into histological subtypes of esophageal cancer and localizations of gastric cancer is important, as this allows the evaluation of different risk factors for the cancer subtypes and more precise estimates of the strength of the associations. Our study is also one of the largest prospective cohort studies investigating this topic.

The ranges of the participants' alcohol consumption and cigarette smoking habits were wide, which gave good contrasts between high and low exposures. Also, alcohol consumption and cigarette smoking habits were addressed extensively in the questionnaire. We lack data on smoking and alcohol consumption after baseline, but we did ask participants about their habits during a long period before baseline. As the development of a tumor probably takes several decades, we believe we asked about the exposure in a relevant time window.

Participants could have changed their alcohol consumption and smoking habits due to preclinical cancer, which may influence results. This is unlikely though, because the smoking data reflect the lifetime exposure of the participants. As for alcohol, results were robust for restriction of analyses to stable users.

In this epidemiological study, we analyze the possibility of residual confounding, by, for example, *Helicobacter pylori* infection. This infection is associated with increased risk of GNCA,²⁹ and possibly with decreased risk of EAC,^{30,31} while it is not associated with GCA²⁹ or ESCC risk.³¹ Unfortunately, we lack data on *H pylori* infection, but we estimate the prevalence to have been ~50%.³² The infection might have confounded the associations if it is correlated with alcohol consumption or smoking. However, studies investigating this correlation have been inconsistent.³³⁻³⁵ Residual confounding by other variables cannot be completely ruled out, but the associations found were strong and residual confounding probably cannot explain these associations entirely. Moreover, the true associations may be stronger than observed, because we probably underestimated their strength due to random measurement error.

Comparison with previous research: alcohol

The strong positive association between alcohol consumption and ESCC risk we found is in agreement with associations found in the other prospective cohorts.^{9,10,36} The association we found showed a statistically significant trend ($p < 0.001$), although low consumers of alcohol were found to be at slightly decreased ESCC risk, and this association remained when we restricted our analyses to the group of stable alcohol consumers. These findings are consistent with findings from two other cohorts,^{10,36} and an explanation was suggested by Freedman et al.¹⁰ Possibly, some of the subjects who were abstainers at baseline used to be heavy drinkers long (>5 years) before baseline. These drinkers remain at increased risk for a decade after cessation of alcohol consumption.³⁷

Our findings that alcohol was not associated with EAC, GCA and GNCA confirm the null results found in two other Western cohorts.^{9,10}

To investigate whether substances in alcoholic beverages other than ethanol are relevant for cancer risk, we adjusted analyses of these beverages for total ethanol intake. This approach has not been used for esophageal or gastric cancers before and gave some new insights. For the beverages, ESCC risk was lower compared with the association with ethanol. This suggests that ethanol is probably the key substance in these beverages, and this suggestion is strengthened by the fact that associations have been found across the world, for many different alcoholic beverages.^{10,38,39} Still, other substances in alcoholic beverages might be relevant. For instance, flavonoids⁴⁰ in wine might explain the inverse association we found with ESCC. This inverse association has also been found by a case-control study,⁴¹ but this association was unadjusted for ethanol intake. Regarding beer, N-nitroso compounds present in this beverage may be partly responsible for the positive associations with ESCC and GNCA.⁴²

Comparison with previous research: cigarette smoking

We found that cigarette smoking was associated with an increased risk of all esophageal and gastric cancer subtypes. For these cancer subtypes, we are the first cohort study to analyze the association with all kinds of smoking aspects. Our findings are consistent with previous reviews.⁴³⁻⁴⁵ It appeared that smoking frequency as well as duration were important in terms of increasing a person's risk. Therefore, the number pack-years smoked may be a good indicator of a person's total exposure.

The relationships we found between smoking and esophageal cancer types were weaker compared with a previous case-control⁴⁵ and two cohort^{10,11} studies, but similar to the results of a third cohort study.⁹ There may be several reasons why other studies found stronger associations: under-reporting of smoking habits in case-control studies, no mutual adjustment for smoking aspects, or no or insufficient adjustment for confounding (e.g., by alcohol consumption). Our RRs might be attenuated because some smokers may have stopped smoking after baseline, but this is unlikely because after smoking cessation people stay at risk for a long period.

Our results showed that smoking cessation decreased risks of all four cancers, but only after one to two decades and these results are in agreement with two reviews.^{43,46}

Interaction

This is the first prospective study to investigate the interaction between alcohol consumption and cigarette smoking and the risk of ESCC. An interaction for ESCC has been established by previous case-control studies (see, for example,^{4,41,47,48}). We confirm this interaction, although this was not statistically significant, probably due to low power. Yet, for EAC, GCA and GNCA it was uncertain whether a similar interaction is present, as only one study examined this.¹⁰ According to our findings, no interaction was present.

The interaction between alcohol consumption and cigarette smoking can be explained biologically. Cigarette smoke contains many carcinogens, and the ethanol from alcoholic beverages is metabolized to acetaldehyde, which was classified as human carcinogen by the International Agency for Research on Cancer (IARC).⁴⁹ Acetaldehyde can circulate in the blood after formation in the liver, but can also be formed locally by oral bacteria.⁵⁰ Ethanol itself can cause local irritation of the upper gastrointestinal tract⁵¹ and may facilitate the uptake of the carcinogens present in cigarette smoke.⁵⁰

In conclusion, alcohol and cigarette smoke each have an individual effect on ESCC risk, but, when combined, they act synergistically. We found no interaction for EAC, GCA, or GNCA.

Conclusions and recommendations

In summary, we found alcohol to be positively associated with ESCC risk, but not with EAC, GCA or GNCA. Cigarette smoking was positively associated with risk of all four cancer types. Alcohol consumption and smoking interacted in a multiplicative way on the risk of ESCC.

Alcohol consumption cannot explain the previously mentioned rising incidences of EAC and GCA, because it was not associated with risk of either. Cigarette smoking habits cannot explain the increasing trends either, because these habits have not increased over time in Western countries. Therefore, we suggest that further research should focus on other risk factors for esophageal and gastric cancer subtypes, to search for explanations for these increases.

Supplemental Table 6.1 Association (age- and sex-adjusted incidence rate ratios) between alcohol consumption and risk of esophageal and gastric cancer subtypes; Netherlands Cohort Study on diet and cancer (NLCS) 1986-2002.

Subcohort		Esophageal squamous cell carcinoma		Esophageal adenocarcinoma		Gastric cardia adenocarcinoma		Gastric noncardia adenocarcinoma	
category	person time at risk (years)	no.	RR ^a (95% CI) ^a	no.	RR (95% CI)	no.	RR (95% CI)	no.	RR (95% CI)
median		cases		cases		cases		cases	
Alcohol consumption (grams ethanol/day)									
Abstainer	13336	16	1	reference	25	1	reference	29	1
>0<5	16740	15	0.78	0.38-1.60	38	1.13	0.68-1.89	30	0.76
5<15	12714	23	1.66	0.86-3.20	30	0.90	0.52-1.57	47	1.13
15<30	8979	24	2.51	1.29-4.87	31	1.13	0.65-1.98	36	1.02
>=30	5037	29	5.52	2.77-11.00	21	1.24	0.67-2.27	22	0.99
			p-trend ^b <0.001			p-trend 0.50			p-trend 0.63
continuous, 10 g ethanol/day increments									
overall	56806	107	1.34	1.22-1.46	145	1.05	0.95-1.16	164	1.00
men	26679	59	1.30	1.18-1.42	114	1.04	0.94-1.16	140	1.01
women	30128	48	1.69	1.42-2.02	31	1.16	0.89-1.52	24	0.88
			p-interaction ^d 0.01			p-interaction 0.51			p-interaction 0.67
Alcohol consumption (grams ethanol/day)									
Stable users ^e									
abstainer	10763	13	1	reference	21	1	reference	23	1
>0<5	9780	8	0.71	0.29-1.73	19	0.86	0.46-1.62	12	0.48
5<15	7831	13	1.53	0.69-3.37	19	0.83	0.44-1.58	22	0.80
15<30	4932	14	2.77	1.25-6.17	18	1.10	0.57-2.14	19	0.93
>=30	2735	15	5.47	2.42-12.35	15	1.55	0.77-3.12	10	0.81
			p-trend <0.001			p-trend 0.11			p-trend 0.62
continuous, 10 grams ethanol/day increments	36041	63	1.40	1.27-1.55	92	1.12	0.99-1.26	86	1.00
									p-trend 0.81
									p-trend 0.92-1.10

	Subcohort		Esophageal squamous cell carcinoma			Esophageal adenocarcinoma			Gastric cardia adenocarcinoma			Gastric noncardia adenocarcinoma			
	categorical median	person time at risk (years)	no.	RR ^a	(95% CI) ^a	no.	RR	(95% CI)	no.	RR	(95% CI)	no.	RR	(95% CI)	
															cases
Alcoholic beverages (glasses/day) ^f															
Beer	no beer	0	38324	60	1	reference	79	1	reference	86	1	reference	294	1	reference
	>0-1	0.2	14606	28	0.96	0.55-1.69	48	0.84	0.55-1.28	59	0.86	0.60-1.25	140	0.89	0.69-1.13
	>1-2	1.4	2543	8	1.35	0.57-3.16	10	0.90	0.44-1.82	12	0.91	0.47-1.77	30	1.10	0.70-1.72
	>2	3.7	1333	11	2.12	0.85-5.30	8	1.28	0.55-2.97	7	1.01	0.45-2.28	27	1.91	1.15-3.15
						p-trend 0.06			p-trend 0.48			p-trend 0.89			p-trend 0.01
continuous, 1 glass/day increments			56806	107	1.15	0.98-1.34	145	1.04	0.87-1.23	164	1.06	0.89-1.26	491	1.20	1.07-1.34
Wine	no wine	0	26060	50	1	reference ^g	74	1	reference	84	1	reference	268	1	reference
	>0-1	0.2	23467	42	0.78	0.51-1.20	55	0.84	0.58-1.21	64	0.89	0.63-1.26	169	0.71	0.58-0.88
	>1-2	1.4	4806	8	0.45	0.20-1.01	11	0.75	0.38-1.45	9	0.62	0.29-1.33	36	0.72	0.48-1.09
	>2	2.7	2473	7	0.27	0.07-1.06	5	0.54	0.19-1.52	7	0.83	0.34-2.01	18	0.62	0.34-1.11
						p-trend 0.03			p-trend 0.21			p-trend 0.42			p-trend 0.12
continuous, 1 glass/day increments			56806	107	0.67	0.50-0.89 ^c	145	0.81	0.60-1.08	164	0.82	0.60-1.13	491	0.82	0.68-1.00
Liquor	no liquor	0	29359	41	1	reference ^c	60	1	reference	60	1	reference	230	1	reference
	>0-1	0.2	20833	31	1.01	0.62-1.64	53	0.94	0.63-1.1	69	1.14	0.79-1.65	183	0.91	0.73-1.13
	>1-2	1.9	4714	21	1.96	1.03-3.72	20	1.19	0.65-2.18	29	1.54	0.87-2.73	59	0.92	0.64-1.34
	>2	2.8	1900	14	1.53	0.59-3.95	12	1.72	0.74-4.03	6	0.76	0.25-2.34	19	0.65	0.36-1.17
						p-trend 0.08			p-trend 0.21			p-trend 0.52			p-trend 0.39
continuous, 1 glass/day increments			56806	107	1.20	0.89-1.63	145	1.15	0.89-1.50	164	1.10	0.81-1.50 ^c	491	0.95	0.80-1.13

^a CI, confidence interval; RR, rate ratio; ^b Tests for dose-response trends were assessed by fitting ordinal variables as continuous terms in the Cox proportional hazard model; ^c Proportional hazards assumption was violated for the exposure variable, and there was a statistically significant interaction with time; ^d p-value for interaction between sex and alcohol consumption, based on cross product term in the Cox proportional hazard model; ^e subjects who had not changed their alcohol consumption habits in the five years before baseline; ^f additionally adjusted for ethanol intake; ^g proportional hazards assumption was violated for the exposure variable, but there was no statistically significant interaction with time.

Supplemental Table 6.2
Association (age- and sex-adjusted incidence rate ratios) between cigarette smoking and risk of esophageal and gastric cancer subtypes; Netherlands Cohort Study on diet and cancer (NLCS) 1986-2002.

categorical median	person time at risk (years)	Subcohort	Esophageal squamous cell carcinoma			Esophageal adenocarcinoma			Gastric cardia adenocarcinoma			Gastric noncardia adenocarcinoma		
			no. cases	RR ^a	(95% CI) ^a	no. cases	RR	(95% CI)	no. cases	RR	(95% CI)	no. cases	RR	(95% CI)
Smoking status														
never smokers	21958		22	1	reference	28	1	reference	28	1	reference	113	1	reference
former smokers	20271		31	1.58	0.85-2.95	70	1.45	0.92-2.29	80	1.35	0.84-2.17	201	1.41	1.08-1.85
current smokers	14577		54	4.09	2.34-7.14	47	1.57	0.96-2.55	56	1.54	0.94-2.51	177	1.95	1.49-2.55
					p-trend ^b <0.001			p-trend 0.09			p-trend 0.09			p-trend <0.001
Frequency of cigarette smoking (no./day)														
no cigarette smoker	21958		22	1	reference	28	1	reference	28	1	reference	113	1	reference
>0-<20	21933		44	2.27	1.27-4.04	50	1.12	0.69-1.81	74	1.33	0.83-2.14	222	1.58	1.22-2.04
>=20	12916		41	3.88	2.09-7.21	67	2.25	1.39-3.63	62	1.59	0.97-2.61	156	1.79	1.34-2.40
					p-trend <0.001			p-trend <0.001			p-trend 0.07			p-trend <0.001
continuous, 10 cigarettes/day increments														
overall	56806		107	1.43	1.26-1.63	145	1.32	1.17-1.48	164	1.13	1.00-1.28	491	1.16	1.07-1.25
men	26679		59	1.25	1.07-1.47	114	1.34	1.18-1.51	140	1.13	0.99-1.28	331	1.14	1.05-1.24
women	30128		48	1.96	1.57-2.44	31	1.13	0.73-1.76	24	1.17	0.78-1.78	160	1.24	1.04-1.48
					p-interaction ^c 0.001			p-interaction 0.40			p-interaction 1.00			p-interaction 0.42
Duration of cigarette smoking (years)														
no cigarette smoker	21958		22	1	reference	28	1	reference	28	1	reference	113	1	reference
>0-<20	6623		4	0.73	0.24-2.21	13	1.03	0.52-2.01	16	1.07	0.55-2.08	52	1.39	0.96-2.00
20-<40	16850		33	2.41	1.31-4.44	61	1.68	1.06-2.67	59	1.34	0.83-2.18	153	1.47	1.11-1.95
>=40	11376		48	4.82	2.64-8.80	43	1.51	0.90-2.53	61	1.72	1.05-2.72	173	2.06	1.56-2.72
					p-trend <0.001			p-trend 0.04			p-trend 0.02			p-trend <0.001
continuous, 10 years increments	56806		107	1.41	1.24-1.61	145	1.13	1.02-1.26	164	1.15	1.03-1.27	491	1.17	1.11-1.25

	categorical median	Subcohort		Esophageal squamous cell carcinoma		Esophageal adenocarcinoma		Gastric cardia adenocarcinoma		Gastric noncardia adenocarcinoma	
		person time at risk (years)	no. cases	RR ^a (95% CI) ^a	no. cases	RR (95% CI)	no. cases	RR (95% CI)	no. cases	RR (95% CI)	
Pack-years of cigarette smoking											
no cigarette smoker	0	21958	22	1	reference	28	1	reference	28	1	reference
>0-<20	9	18505	25	1.62	0.86-3.03	38	1.05	0.63-1.75	52	1.17	0.71-1.94
20-<40	28	11247	39	4.53	2.41-8.48	42	1.65	0.99-2.76	55	1.68	1.01-2.78
>=40	48	5096	21	4.99	2.47-10.05	37	2.98	1.74-5.11	29	1.78	1.01-3.13
				p-trend <0.001			p-trend <0.001			p-trend 0.01	p-trend <0.001
continuous, 10 pack-years increments											
overall		56806	107	1.27	1.18-1.37	145	1.18	1.10-1.27	164	1.09	1.02-1.17
men		26679	59	1.20	1.10-1.31	114	1.19	1.10-1.28	140	1.09	1.02-1.17
women		30128	48	1.50	1.32-1.69	31	1.14	0.88-1.46	24	1.08	0.83-1.40
				p-interaction 0.004			p-interaction 0.66			p-interaction 0.81	p-interaction 0.64
Smoking cessation											
never smokers		21958	22	1	reference	28	1	reference	28	1	reference
stopped >= 20 years	25	6280	10	1.60	0.71-3.62	19	1.21	0.66-2.22	18	0.93	0.49-1.74
stopped 10-<20 years	14	6953	10	1.50	0.69-3.27	23	1.38	0.78-2.43	28	1.36	0.78-2.37
stopped >0-<10 years	5	6984	11	1.67	0.74-3.75	28	1.77	1.03-3.03	34	1.75	1.01-3.03
current smokers	0	14577	54	4.10	2.35-7.15	47	1.56	0.96-2.55	56	1.54	0.94-2.51
				p-trend <0.001			p-trend 0.04			p-trend 0.02	p-trend <0.001

^a CI, confidence interval; RR, rate ratio; ^b Tests for dose-response trends were assessed by fitting ordinal variables as continuous terms in the Cox proportional hazard model; ^c p-value for interaction between sex and cigarette smoking frequency, based on cross product term in the Cox proportional hazard model.

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7

Selenium status and the risk of esophageal and gastric cancer subtypes: the Netherlands Cohort Study

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ABSTRACT

Background & Aims

Selenium may protect against the development of esophageal squamous cell carcinoma (ESCC), esophageal adenocarcinoma (EAC) and gastric cardia adenocarcinoma (GCA). Only in very few studies have the associations with ESCC and GCA been investigated, and no epidemiologic studies exist on EAC.

Methods

We studied the association between selenium and risk of ESCC, EAC, and GCA within the prospective Netherlands Cohort Study, conducted among 120,852 men and women aged 55-69 years at baseline. In September 1986, the cohort members completed a questionnaire on risk factors for cancer and provided toenail clippings for determination of baseline selenium status. After 16.3 years of follow-up, 64 ESCC, 112 EAC, and 114 GCA cases and 2072 subcohort members were available for case-cohort analysis. Incidence rate ratios (RR) were calculated using Cox proportional hazards models.

Results

In multivariable analyses of selenium status, we found an inverse association with ESCC ($RR_{\text{per standard unit increment}} 0.80$, 95% confidence interval (CI) 0.67-0.96), and a borderline significant inverse association with GCA ($RR 0.91$, 95% CI 0.80-1.02). No overall association was observed for EAC ($RR 1.05$, 95% CI 0.95-1.15), but for women and never smokers, significant inverse associations were found ($RR_{\text{per standard unit increment}} 0.72$, 95% CI 0.61-0.84 and 0.74, 95% CI 0.64-0.86, respectively).

Conclusions

This prospective study supports an inverse association between toenail selenium and risk of ESCC and GCA and suggests an inverse association with risk of EAC in subgroups (women, never smokers, and low antioxidant consumers). These associations need confirmation.

INTRODUCTION

Selenium has been studied in relation to cancer risk, and most epidemiologic studies have been performed on breast, colorectal, lung, gastric, bladder, and prostate cancer.¹ Evidence from observational studies so far suggests that selenium is inversely associated with prostate cancer, and possibly with lung and gastric cancer.¹ The reason for the interest in selenium in relation to cancer etiology is the antioxidant capacity of the selenium-dependent glutathione peroxidase enzymes.^{2,3} Other possible mechanisms through which selenium could be associated with lower cancer risk include reduction of inflammation, induction of detoxifying phase II enzymes, increase of p53, alteration of DNA methylation, blockage of the cell cycle, induction of apoptosis of cancer cells and inhibition of angiogenesis.³

The mineral is an essential trace element present in food. The selenium content of foods depends on the selenium content of the soil on which the food is grown. Regions with very low soil selenium levels (<0.05 ppm) are New Zealand, parts of China, and Finland, while high levels (>5 ppm) are found in Canada, parts of the western United States, parts of China, Ireland, France, and Germany.⁴ The recommended daily allowance for selenium is 55 µg/day in the United States⁵, and 50-150 µg/day in The Netherlands.⁶ For the Dutch population, the most important sources of selenium are meat, bread, milk, and milk product, fish, eggs, and cheese.⁷ Generally high serum selenium levels are reported in the United States, whereas in The Netherlands, blood and toenail selenium levels are intermediate between those reported from New Zealand and the United States.⁸ In epidemiologic research, the measurement of selenium status through biomarkers is preferred over the measurement of selenium intake through questionnaires because of the high variability of food selenium content.⁹ Toenails are a suitable biomarker because they reflect the intake of selenium for a period up to 1 year.^{10,11}

Data on possible associations between selenium and esophageal and gastric cancer are sparse, and even less is known about the subtypes of these cancers. A few epidemiologic studies¹²⁻¹⁶ of different design found indications for an inverse association between selenium and total esophageal cancer. Limited evidence suggestive of an inverse association with esophageal squamous cell carcinoma (ESCC) arises from studies in Chinese populations.¹⁷⁻²⁰ We could not find any case-control or cohort studies on selenium and esophageal adenocarcinoma (EAC) risk, but 1 US cross-sectional study found an inverse association between serum selenium and markers of neoplastic progression in Barrett's esophagus cases (a precursor lesion of EAC).²¹ Selenium and its association with gastric cancer risk has been described in a review,²² but most studies, including ours,²³ did not study the cardia specifically. In our case, this was due to the limited number of cases after 3.3 years of follow-up of our cohort. A cohort study¹⁷ and trial²⁴ in Linxian, China, studying gastric cardia adenocarcinoma (GCA) specifically, were able to identify an inverse association.

It is important to study subtypes of esophageal and gastric cancer separately in analysis because these probably differ with regard to their etiology.^{25,26} EAC and GCA are specifically of interest because the incidences of these cancers have risen strongly in the United States and Europe during the past decades.^{27,28}

The purpose of this study was to prospectively investigate the association between prediagnostic toenail selenium levels and risk of ESCC, EAC, and GCA. For this investigation, we used 16.3-year follow-up data from The Netherlands Cohort Study on diet and cancer (NLCS).

Our hypothesis was that toenail selenium levels are inversely associated with risk of ESCC, EAC, and GCA. The association with ESCC was expected to be the strongest because ESCC is strongly associated with smoking, which causes oxidative stress. This oxidative stress may be counteracted by the antioxidant capacity of selenium.

We studied the potential interaction between selenium and cigarette smoking, intake of vitamins C, E, and several carotenoids. These factors were chosen because they may all be involved in an antioxidant mechanism,^{3,29} and evidence suggestive of these interactions was previously found for total and prostate cancer.^{30,31} Additionally, potential interaction with body mass index (BMI) was investigated because of its association with esophageal cancer.

PATIENTS AND METHODS

Study design and participants

In September 1986, the prospective NLCS was initiated when 58,279 Dutch men and 62,573 women aged 55-69 years were enrolled. The subjects were randomly selected from 204 Dutch municipal registries. At baseline, all cohort members completed a self-administered questionnaire and were asked to provide toenail clippings. A detailed description of the study was previously published.³²

The case-cohort approach is used for data processing and analysis, for reasons of efficiency.³³ Cases are derived from the entire cohort. A subcohort of 3500 subjects was selected at random from the total cohort at baseline. These 3500 subjects were used for the estimation of person-years at risk in the total cohort. Person-years at risk were calculated from the start of the study until esophageal or gastric cancer diagnosis, death, emigration, loss to follow-up or end of follow-up (December 31, 2002), whichever occurred first. Follow-up of the subcohort was established by linkage to Dutch municipal population registries and after 16.3 years (September 1986 to December 2002), only 1 male subcohort member was lost to follow-up. We excluded subcohort members (n=154) who reported at baseline to have prevalent cancer (other than skin cancer), leaving 3346 subcohort members.

The entire cohort was linked to The Netherlands Cancer Registry and the nationwide network and registry of histopathology and cytopathology in The

Netherlands³⁴ for cancer incidence.³⁵ Follow-up for cancer incidence was $\geq 96\%$ complete,³⁶ and after 16.3 years, the following numbers of incident, microscopically confirmed, primary carcinomas were identified: 130 ESCC³⁷ [International Classification of Diseases (ICD) for Oncology (O)-3, C15], 181 EAC³⁷ (C15), and 206 GCA (C16.0). The Medical Ethics Committee of Maastricht University, The Netherlands, has approved the study.

Exposure data

At baseline, all cohort members completed a self-administered questionnaire. The questionnaire consisted of a 150-item food frequency questionnaire and other questions on cancer risk factors, eg, smoking habits, alcohol consumption, height, and weight. The food frequency questionnaire asked about habitual consumption in the year preceding the start of the study. Mean daily nutrient intakes were calculated using the Dutch food-composition table.³⁸ The questionnaire data were key entered and processed in a standardized manner, blinded with respect to cases/subcohort status to minimize observer bias in coding and interpretation of the data.

In our cohort, about 75% of the subjects provided toenail clippings. Toenail selenium determinations were carried out by the Reactor Institute Delft (Delft University of Technology, Delft, The Netherlands). The determination was based on instrumental neutron activation analysis of the ^{77m}Se isotope (metastable selenium-77 isotope, half-life 17.5 seconds). Each sample went through 6 cycles of 17-second irradiation at a thermal neutron flux of $3 \times 10^{16} \text{ m}^{-2} \text{ s}^{-1}$, 3-second decay, and 17-second counting at 1 cm from a 40% germanium detector. This method and the use in the NLCS have been described in more detail previously.^{23,39-41}

In 1992, the toenail selenium determinations for the subcohort members and gastric cancer cases diagnosed until 3.3 years of follow-up (until December 31, 1990) were carried out. In 2008, the selenium determinations were carried out for all esophageal cancer cases and the gastric cardia cancer cases occurring from 3.3-16.3 years of follow-up (December 31, 1990, until December 31, 2002). In 1992, the "SBP" (abbreviation of Snelle Buizen Post, Dutch for fast pneumatic system) facility was used for instrumental neutron activation analysis, and, since 1996, the "CAFIA" (Carbonfiber Autonomous Facility for Irradiation and Analysis) facility has been used. To assess the validity and comparability of these 2 methods, the toenail selenium levels for 40 subcohort members were assessed in 1996 with the "CAFIA" facility in addition to the original assessment with the "SBP" facility.³⁹ The mean selenium level $\pm$ standard deviation (SD) assessed by the "CAFIA" facility ($0.552 \pm 0.05 \text{ } \mu\text{g/g}$) was comparable with mean selenium levels assessed by the "SBP" facility ($0.551 \pm 0.04 \text{ } \mu\text{g/g}$) for these subjects. The Pearson correlation coefficient between toenail selenium levels assessed by the "CAFIA" facility and those estimated by the "SBP" facility was 0.95 ($p < 0.01$).³⁹ It was concluded that both methods were valid and comparable.

In 1992, all toenail clippings provided by cases and subcohort were sent to the Reactor Institute Delft for selenium determination. This determination, however, yields unreliable results if the nail samples weigh <10 mg, and these measurements were thus excluded. In 2007, we discovered that toenail clippings can also be used as a source of DNA⁴². We therefore separated and saved 10-20 mg of toenail clippings from the cancer cases for future genetic research. If, afterwards, more than 10 mg of toenail clippings was left, we sent them to the Reactor Institute Delft. In case there were problems with the determination of toenail selenium due to very high calcium contents, the subject was excluded. Complete selenium data were available for 2426 subcohort members, and 71 ESCC, 129 EAC, and 127 GCA cases.

Statistical analysis

To evaluate the potential influence of prediagnostic cancer at baseline on toenail selenium levels, cases were categorized according to the year of follow-up in which they were diagnosed. Mean toenail selenium levels of ESCC, EAC and GCA cancer cases were compared according to year of follow-up, and differences were tested using a t-test. For the t-test, selenium levels were $\ln$ (natural logarithm)-transformed to normalize the distribution. For the case-cohort analyses, toenail selenium levels were categorized into quartiles according to the distribution in the subcohort. For continuous analyses, the selenium levels were standardized to the average size of the 2 central quartiles in the subcohort. One standardized selenium unit is equal to 0.06 $\mu\text{g/g}$.

Excluded were subcohort members and cases with inconsistent or incomplete dietary questionnaire data⁴³ and those with missing data on the confounders. Complete data for statistical analysis was available for 2072 subcohort members, and 64 ESCC, 112 EAC, and 114 GCA cases.

The following variables were considered confounders because of their established association with esophageal or gastric cancer:⁴⁴ age, sex, cigarette smoking (current yes/no, number of cigarettes smoked daily, and number of smoking years), alcohol consumption (g/day), and BMI (kg/m^2). The following variables were considered potential confounders but were eventually not included in the models because they did not change the incidence rate ratio (RR) by >5%: nonoccupational physical activity; highest level of education; daily intakes of vitamin C, vitamin E, α -carotene, β -carotene, β -cryptoxanthin, lycopene, and lutein/zeaxanthin; family history of esophageal or gastric cancer; reported long-term (>0.5 years) use of nonsteroidal anti-inflammatory drugs or aspirin (Anatomical therapeutic chemical (ATC) codes M01A, N02B); or lower esophageal sphincter relaxing medication.⁴⁵⁻⁴⁷

Cox proportional hazards models were used to estimate age- and sex-adjusted and multivariable adjusted incidence RRs and corresponding 95% confidence intervals (CI).⁴⁸ Analyses were done using Stata 9.2 statistical software package (StataCorp, College Station, Texas, USA). Standard errors were estimated using the robust Huber-

White sandwich estimator to account for additional variance introduced by sampling from the cohort. This method is equivalent to the variance-covariance estimator by Barlow.⁴⁹ The proportional hazards assumption was tested using the scaled Schoenfeld residuals,⁵⁰ and -log(-log) plots. Tests for dose-response trends were assessed by fitting ordinal exposure variables as continuous terms. Two-sided p-values are reported throughout the article.

Because of the limited numbers of cases, all analyses were carried out for both sexes combined. Nevertheless, tests for interaction between toenail selenium and sex were performed using the continuous selenium variable. We only report results stratified by sex for the continuous selenium variable.

We investigated possible interactions between toenail selenium status and cigarette smoking status (current, former, never), BMI, and intakes of several vitamins and carotenoids by estimating RRs in strata of these exposures. The p-value for interaction was assessed by including a cross-product term in the model. In the interaction analyses, we used a continuous selenium variable, because of the limited numbers of cases. To evaluate potential bias introduced by prediagnostic cancer at baseline, which may influence toenail selenium levels, we performed sensitivity analyses, excluding cases from the first 2 years of follow-up.

RESULTS

Table 7.1 shows that, when the cases were categorized according to 2-year follow-up periods in which they were diagnosed, there was no clear trend towards lower toenail selenium levels in cases occurring during early follow-up, indicating no effect of preclinical disease on toenail selenium levels. The t-tests were also not statistically significant.

The median toenail selenium level (in micrograms/grams) of all subcohort members was 0.552. The levels were lower in men (0.538) than in women (0.564). For the cases, the following median toenail selenium levels (in $\mu\text{g/g}$) were observed: ESCC, 0.493; EAC, 0.532; and GCA, 0.529 (Table 7.2).

Table 7.2 presents some characteristics of the subcohort members and ESCC, EAC, and GCA cases. The most salient differences are described hereafter. There were far more men than women among the EAC and GCA cases. Among the subcohort members, there were relatively more never cigarette smokers, and subcohort members had, on average, higher intakes of vitamins C and E, and carotenoids than the cases. With respect to separate cancer groups, we found that ESCC cases consumed much more alcohol and more often had a low level of education compared with the subcohort. EAC cases had a higher BMI, and more often reported a family history of esophageal or gastric cancer and use of lower esophageal sphincter relaxing medication. GCA cases spent more time on nonoccupational physical activity than the subcohort.

Table 7.1 Toenail selenium levels ($\mu\text{g/g}$) in esophageal and gastric cancer cases according to sex and time between baseline and esophageal or gastric cancer diagnosis; Netherlands Cohort Study (1986-2002).

Cases ^a	Esophageal squamous cell carcinoma				Esophageal adenocarcinoma				Gastric cardia adenocarcinoma			
	No. cases	Toenail selenium level ($\mu\text{g/g}$)		p^b	No. cases	Toenail selenium level ($\mu\text{g/g}$)		p^b	No. cases	Toenail selenium level ($\mu\text{g/g}$)		p^b
		mean	sd			mean	sd			mean	sd	
All cases	71	0.514	0.088		129	0.564	0.163		127	0.535	0.080	
Men	44	0.505	0.092		106	0.571	0.177		109	0.536	0.082	
Women	27	0.527	0.081		23	0.533	0.049		18	0.532	0.071	
Year of follow-up												
0-2	4	0.462	0.103	0.20	8	0.514	0.065	0.30	12	0.537	0.085	0.96
>2-4	9	0.568	0.144		12	0.549	0.070		17	0.573	0.087	
>4-6	8	0.446	0.051		15	0.617	0.296		11	0.534	0.093	
>6-8	11	0.509	0.072		20	0.590	0.162		17	0.534	0.082	
>8-10	9	0.535	0.090		14	0.537	0.081		18	0.509	0.069	
>10-12	11	0.531	0.077		20	0.612	0.244		24	0.550	0.077	
>12-14	7	0.523	0.058		19	0.515	0.063		12	0.538	0.083	
>14-17	12	0.503	0.066		21	0.547	0.064		16	0.502	0.061	

^a Mean $\pm$ sd selenium levels in subcohort members were $0.547 \pm 0.126 \mu\text{g/g}$ for men ($n=1211$) and $0.575 \pm 0.109 \mu\text{g/g}$ for women ($n=1248$); ^b T-test of mean toenail selenium level (ln-transformed) in first 2 years of follow-up versus rest of follow-up years.

Table 7.3 reports on the association between quartiles of toenail selenium levels and risk of ESCC, EAC, and GCA. For ESCC, an inverse association was found with toenail selenium (multivariable RR for the highest vs the lowest quartile 0.37, 95% CI 0.16-0.86), and the test for trend was statistically significant ($p\text{-trend}=0.02$). The association was nearly the same in age- and sex-adjusted analyses, and the results were also robust to the exclusion of the first 2 years of follow-up.

No clear association was found between toenail selenium levels and EAC risk (multivariable RR for the highest vs the lowest quartile 0.76, 95% CI 0.41-1.40). The results from age- and sex-adjusted analyses and analyses excluding the first 2 years of follow-up showed no association either. However, a statistically significant ($p<0.001$) interaction was found between toenail selenium and sex: an inverse association was observed in women, but not in men (Table 7.3). As mentioned before, we analyzed men and women together in further analyses because of the small number of cases.

Table 7.2 Characteristics of cases and subcohort members in the Netherlands Cohort Study (1986-2002).

Exposure variables and potential confounders	Subcohort	Cases		
	(n=2072) ^a	ESCC ^b (n=64) ^a	EAC ^b (n=112) ^a	GCA ^b (n=114) ^a
	median (IQR)	median (IQR)	median (IQR)	median (IQR)
Toenail selenium level (µg/g)				
total	0.552 (0.498-0.613)	0.493 (0.453-0.583)	0.532 (0.488-0.584)	0.529 (0.477-0.582)
men	0.538 (0.483-0.602)	0.490 (0.448-0.558)	0.536 (0.485-0.588)	0.529 (0.477-0.581)
women	0.564 (0.514-0.623)	0.524 (0.473-0.597)	0.516 (0.501-0.564)	0.518 (0.485-0.583)
	mean (sd) ^c	mean (sd)	mean (sd)	mean (sd)
Age at baseline (y)	61.2 (4.2)	62.2 (4.0)	61.5 (4.1)	61.3 (4.2)
Sex				
Men (n (%))	1020 (49)	40 (63)	93 (83)	97 (85)
Women (n (%))	1052 (51)	24 (37)	19 (17)	17 (15)
Cigarette smoking status				
Never smoker (n (%))	782 (38)	11 (17)	19 (17)	15 (13)
Former smoker (n (%))	760 (37)	21 (33)	61 (54)	57 (50)
Current smoker (n (%))	530 (25)	32 (50)	32 (29)	42 (37)
Ever cigarette smokers				
Frequency of cigarette smoking (no./d)	15 (10)	17 (10)	21 (14)	17 (11)
Duration of cigarette smoking (y)	31 (12)	37 (10)	34 (11)	35 (11)
Ethanol intake (g/d)	10 (14)	24 (29)	15 (18)	13 (14)
Body mass index (BMI) (kg/m ²)	25.0 (3.1)	23.9 (3.0)	26.4 (3.3)	25.5 (2.6)
Nonoccupational physical activity (min/d)	73 (58)	74 (62)	77 (58)	89 (82)
Highest level of education ^d				
Primary (n (%))	565 (27)	23 (36)	25 (23)	26 (23)
Lower vocational (n (%))	457 (22)	12 (19)	26 (24)	34 (30)
Secondary and medium vocational (n (%))	754 (37)	21 (33)	40 (36)	32 (28)
University and higher vocational (n (%))	283 (14)	8 (12)	19 (17)	22 (19)
Vitamin C intake (mg/d)	104 (42)	100 (47)	98 (50)	90 (40)
Vitamin E intake (mg/d)	14 (6)	13 (5)	14 (6)	14 (7)
α-carotene intake (µg/d)	711 (587)	680 (513)	701 (539)	659 (555)
β-carotene intake (µg/d)	3001 (1600)	2869 (1359)	2970 (1525)	2924 (1597)
β-cryptoxanthin intake (µg/d)	181 (165)	174 (194)	150 (174)	131 (146)
Lycopene intake (µg/d)	1210 (1762)	1056 (1297)	985 (1722)	1067 (1118)
Lutein/zeaxanthin intake (µg/d)	2520 (1096)	2411 (925)	2567 (1140)	2555 (1317)
Family history of esophageal or gastric cancer (n (%))	173 (8)	7 (11)	15 (13)	10 (9)
Reported long-term use of NSAIDs ^b (n (%))	152 (7)	5 (8)	10 (9)	5 (4)
Reported long-term use of LES ^b relaxing medication (n (%))	288 (14)	9 (14)	27 (24)	18 (16)

^a Presented are the number of subcohort members or cases with complete data on toenail selenium level, age, sex, cigarette smoking (current yes/no, number of cigarettes smoked daily, number of smoking years), alcohol consumption and BMI. Subcohort members and cases with incomplete or inconsistent questionnaire data are excluded; ^b ESCC: esophageal squamous cell carcinoma, EAC: esophageal adenocarcinoma, GCA: gastric cardia adenocarcinoma, LES: lower esophageal sphincter, NSAIDs: nonsteroidal anti-inflammatory drugs; ^c For categorical variables n (%) is presented; ^d Numbers do not always add up to the total, because of missing values on this variable.

Table 7.3 Incidence rate ratios of esophageal and gastric cancer subtypes according to toenail selenium levels; Netherlands Cohort Study (1986-2002).

Subcohort	Esophageal squamous cell carcinoma		Esophageal adenocarcinoma		Gastric cardia adenocarcinoma	
	person time at risk (years)	no. cases	RR ^a (95% CI) ^a	no. cases	RR ^a (95% CI) ^a	no. cases
All years of follow-up						
Age- and sex-adjusted analyses						
Quartiles of toenail selenium (boundaries in µg/g)						
1 (≤ 0.498)	7207	33	reference	33	reference	41
2 (≤ 0.552)	7614	10	0.30	35	1.15	27
3 (≤ 0.613)	7705	12	0.37	24	0.85	31
4 (> 0.613)	7584	9	0.28	20	0.74	15
p-value for linear trend ^b			0.001		0.20	0.04
increment in standard units ^c						
men	30110	64	0.72	112	1.03	114
women	14207	40	0.74	93	1.05	97
	15903	24	0.71	19	0.74	17
			p-interaction ^d 0.81		p-interaction ^d <0.001	p-interaction ^d 0.12
Multivariable adjusted analyses^e						
Quartiles of toenail selenium (boundaries in µg/g)						
1 (≤ 0.498)	7207	33	Reference	33	reference	41
2 (≤ 0.552)	7614	10	0.36	35	1.13	27
3 (≤ 0.613)	7705	12	0.45	24	0.84	31
4 (> 0.613)	7584	9	0.37	20	0.76	15
p-value for linear trend ^b			0.02		0.25	0.14
increment in standard units ^c						
men	30110	64	0.80	112	1.05	114
women	14207	40	0.81	93	1.07	97
	15903	24	0.79	19	0.72	17
			p-interaction ^d 0.84		p-interaction ^d <0.001	p-interaction ^d 0.07

Subcohort	Esophageal squamous cell carcinoma		Esophageal adenocarcinoma		Gastric cardia adenocarcinoma	
	person time at risk (years)	no. cases	RR ^a	(95% CI) ^a	no. cases	RR ^a (95% CI) ^a
First 2 years of follow-up excluded						
Multivariable adjusted analyses ^e						
Quartiles of toenail selenium (boundaries in µg/g)						
1 (≤ 0.498)	6190	30	1	reference	38	1
2 (≤ 0.552)	6579	10	0.40	0.19-0.82	24	0.72
3 (≤ 0.613)	6666	11	0.46	0.20-1.01	30	1.01
4 (> 0.613)	6560	9	0.42	0.18-0.96 ^f	14	0.53
p-value for linear trend ^b				0.04		0.21
increment in standard units ^c	25996	60	0.82	0.68-0.98	106	0.92
men	12188	38	0.82	0.64-1.05	90	0.95
women	13808	27	0.82	0.66-1.02	16	0.73
			p-interaction ^d	0.96	p-interaction ^d	0.08

^a RR, Incidence Rate Ratio; 95% CI, 95 percent confidence interval; ^b Tests for dose-response trends were assessed by fitting ordinal variables as continuous terms in the Cox proportional hazard model; ^c For continuous analyses, the selenium levels were standardized to the average size of the two central quartiles. One standardized selenium unit is equal to 0.06 µg/g; ^d p-value for interaction between sex and toenail selenium level, based on cross product term in the Cox proportional hazard model. ^e adjusted for age (years), sex, cigarette smoking (current smoking status (yes/no), frequency (number of cigarettes/day), and duration (years)), alcohol consumption (g/day), body mass index (kg/m²); ^f proportional hazards assumption was violated for selenium.

Table 7.4 Association^a between toenail selenium^b levels and risk of esophageal and gastric cancer subtypes stratified by cigarette smoking status, body mass index and intake levels of antioxidants; Netherlands Cohort Study (1986-2002)

	Subcohort	Esophageal squamous cell carcinoma			Esophageal adenocarcinoma			Gastric cardia adenocarcinoma		
		No. cases	RR ^c	(95% CI) ^c	No. cases	RR ^c	(95% CI) ^c	No. cases	RR ^c	(95% CI) ^c
Cigarette smoking status										
	Never smoker	11745	11	0.73 (0.53-0.99)	19	0.74 (0.64-0.86)	15	0.69 (0.52-0.90)		
	Former smoker	10906	21	0.72 (0.53-0.97)	61	1.07 (0.99-1.15)	57	0.88 (0.73-1.06)		
	Current smoker	7459	32	0.92 (0.71-1.19)	32	1.18 (0.84-1.65)	42	1.10 (0.91-1.33)		
			p-interaction 0.50			p-interaction <0.001		p-interaction 0.01		
Body mass index (kg/m ²)										
	<25	16181	42	0.89 (0.74-1.07)	40	1.03 (0.91-1.17)	49	0.94 (0.81-1.09)		
	≥25	13929	22	0.59 (0.43-0.82)	72	1.10 (0.81-1.49)	65	0.89 (0.74-1.06)		
			p-interaction 0.16			p-interaction 0.64		p-interaction 0.81		
Antioxidant intake										
	Vitamin C intake (mg/d)									
	low (≤97)	14755	38	0.77 (0.58-1.02)	67	0.86 (0.73-1.01)	71	0.85 (0.72-1.12)		
	high (>97)	15355	26	0.81 (0.64-1.01)	45	1.21 (1.04-1.41)	43	0.97 (0.81-1.15)		
			p-interaction 0.37			p-interaction <0.001		p-interaction 0.12		
	Vitamin E intake (mg/d)									
	low (≤12)	14992	29	0.84 (0.62-1.12)	54	1.24 (1.01-1.53)	49	0.94 (0.78-1.13)		
	high (>12)	15117	35	0.77 (0.61-0.96)	58	0.90 (0.77-1.06)	65	0.88 (0.75-1.04)		
			p-interaction 0.50			p-interaction 0.02		p-interaction 0.94		
	α-carotene intake (μg/d)									
	low (≤573)	14779	33	0.78 (0.61-1.00)	59	0.83 (0.70-0.99)	58	0.88 (0.71-1.09)		
	high (>573)	15330	31	0.76 (0.58-0.99)	53	1.08 (1.01-1.16)	56	0.93 (0.81-1.07)		
			p-interaction 0.87			p-interaction 0.01		p-interaction 0.38		
	β-carotene intake (μg/d)									
	low (≤2680)	14903	36	0.76 (0.58-1.01)	58	0.83 (0.69-0.99)	63	0.87 (0.73-1.05)		
	high (>2680)	15207	28	0.82 (0.65-1.02)	54	1.24 (1.07-1.43)	51	0.94 (0.79-1.11)		
			p-interaction 0.91			p-interaction 0.004		p-interaction 0.53		
	β-cryptoxanthin intake (μg/d)									
	low (≤130)	14705	35	0.71 (0.57-0.89)	66	1.00 (0.76-1.31)	71	0.93 (0.81-1.08)		
	high (>130)	15404	29	0.89 (0.69-1.14)	46	1.05 (0.97-1.14)	43	0.86 (0.69-1.07)		
			p-interaction 0.05			p-interaction 0.46		p-interaction 0.63		

Subcohort	Esophageal squamous cell carcinoma			Esophageal adenocarcinoma			Gastric cardia adenocarcinoma		
	person time at risk (years)	No. cases	RR ^c (95% CI) ^c	No. cases	RR ^c (95% CI) ^c	No. cases	No. cases	RR ^c (95% CI) ^c	No. cases
Lycopene intake (µg/d) low (≤823) high (>823)	14943	33	0.91 (0.74-1.12)	71	0.82 (0.70-0.96)	58	58	0.83 (0.69-0.99)	
	15167	31	0.64 (0.48-0.87) p-interaction 0.21	41	1.25 (1.09-1.44) p-interaction <0.001	56	56	0.98 (0.84-1.14) p-interaction 0.21	
Lutein/Zeaxanthin intake (µg/d) low (≤2360) high (>2360)	14956	35	0.86 (0.66-1.11)	60	0.94 (0.68-1.29)	63	63	0.93 (0.78-1.11)	
	15154	29	0.72 (0.57-0.90) p-interaction 0.33	52	1.16 (0.99-1.36) p-interaction 0.20	51	51	0.88 (0.74-1.05) p-interaction 0.86	

^a adjusted for age (years), sex, cigarette smoking (current smoking status (yes/no), frequency (number of cigarettes/day), and duration (years)), alcohol consumption (g/day), body mass index (kg/m²); ^b for continuous analyses, the selenium levels were standardized to the average size of the two central quartiles. One standardized selenium unit is equal to 0.06 µg/g; ^c RR, Incidence Rate Ratio; 95% CI, 95 percent confidence interval; ^d proportional hazards assumption was violated for selenium.

We found an inverse association between toenail selenium levels and risk of GCA (multivariable RR for the highest vs the lowest quartile 0.52, 95% CI 0.27-1.02), although the test for trend was not statistically significant ($p=0.14$). As for EAC, the RRs in women were lower (and statistically significant) than in men, and the interaction between selenium and sex was borderline significant ($p=0.07$). The age- and sex-adjusted results were nearly the same as the multivariable results, but statistical significance was only reached in the former. Exclusion of the first 2 years of follow-up did not alter the results (Table 7.3). Because the t-tests (Table 7.1) and the exclusion of the first 2 years of follow-up indicated that selenium levels were not affected by prediagnostic disease, all following analyses are based on the complete 16.3-year follow-up data.

Presented in Table 7.4 are the results of the interaction analyses. For EAC and GCA, a statistically significant interaction was found between selenium and smoking status: only for never smokers was an inverse association with selenium present. The RRs for 1 standardized selenium unit increment were 0.74 (95% CI 0.64-0.86) for EAC and 0.69 (95% CI 0.52-0.90) for GCA for never smokers. No interaction was found for ESCC (p -interaction=0.50), although an inverse association was more apparent for never and former smokers.

With regard to BMI, no interaction with selenium was observed for ESCC, EAC, or GCA (Table 7.4). From Table 7.4, which presents results stratified by intake of several antioxidants, it can be seen that the inverse association we observed between toenail selenium levels and risk of ESCC did not differ between strata, defined by low or high intake of these antioxidants. However, for EAC we found several statistically significant interactions between selenium and antioxidant intake. For subjects with a low intake of vitamin C, α -carotene, β -carotene, or lycopene, (borderline) significant inverse associations with selenium were present. For GCA, no appreciable differences in associations were found between strata of antioxidant intake.

DISCUSSION

This study is the first prospective cohort study into selenium and risk of subtypes of esophageal and gastric cancer in a Western population, and the first to separately investigate ESCC, EAC, and GCA. Our study provides evidence for an inverse association between toenail selenium levels and risk of ESCC and GCA. The association with GCA was more apparent in women, and only in women was an inverse association found with EAC risk, but this was based on small numbers. We found these results in a Dutch population with a median toenail selenium level of 0.522 $\mu\text{g/g}$ (subcohort). This is a low to moderate toenail selenium level when compared to mean levels found in other populations (general population or control subjects): China (Sichuan province), 0.211 $\mu\text{g/g}$;⁵¹ Finland, 0.47 $\mu\text{g/g}$;⁵² United States, 0.83-0.92 $\mu\text{g/g}$;⁵³ Colombia, 0.945 $\mu\text{g/g}$;⁵⁴ and USA (South Dakota/Wyoming), 1.517 $\mu\text{g/g}$.¹¹

Only 1 case-control study, in a Chinese population with low selenium intake, investigated selenium intake, measured by means of a food frequency questionnaire, in relation to ESCC risk.²⁰ Intake of selenium, measured by questionnaires, correlates poorly with actual selenium intake.⁵⁵ However, the Chinese study was able to find a statistically significant inverse association ($p\text{-trend} < 0.001$).²⁰ In the Chinese Linxian trial cohort,^{17, 18} which is a population with low selenium levels, a significant inverse association between serum selenium and ESCC was also found, and the range of selenium levels in that population was comparable to the range in our cohort.⁸ Two other case-control studies investigated esophageal cancer, but did not make a distinction between ESCC and EAC.^{12,16} However, these esophageal cancer cases were presumably mostly ESCC cases. One of these studies was performed in a Japanese population with a moderate selenium status¹² and the other in an Indian population with a high selenium status.¹⁶ These studies reported significantly lower blood selenium levels in esophageal cancer cases than in controls, but did not report rate ratios or odds ratios. Also, the US Nutritional Prevention of Cancer trial found lower esophageal cancer risk in subjects who received daily selenium supplements.¹⁵ The results of the studies mentioned above suggest inverse associations with ESCC over the range from low to high selenium status, whereas too few studies have been performed to make any inferences on EAC.

For GCA, the Linxian trial cohort reported a statistically significant inverse association with serum selenium.¹⁷ Two other studies, in a Chinese and Finnish population with a relatively low selenium status, found an inverse association between serum selenium levels and total gastric cancer.^{12,30} Within the NLCS, we previously found evidence suggestive of an inverse association with total gastric cancer.²³ In contrast, no inverse association was found between serum or toenail selenium levels and gastric cancer in 3 other studies in Japanese, Hawaiian (with Japanese ancestry) and Colombian populations with a higher selenium status.^{54,56,57} This suggests that a ceiling effect may exist, such that selenium is only associated with gastric cancer risk in populations with relatively low selenium levels.

If selenium is associated with lower esophageal and gastric cancer risk, the question arises by which mechanism selenium exerts its effect and in which stage of carcinogenesis. Two studies, investigating subjects with Barrett's esophagus²¹ and with dysplasia of the squamous epithelium,¹⁹ can give clues about the timing of the effect of selenium. The former study found less signs of progression in subjects with the highest serum selenium levels, whereas the latter found that selenium supplements were associated with less progression and more regression of the dysplasia. These findings may mean that selenium can have an effect after the epithelium of the esophagus has already changed. No effect, however, of selenium containing supplements was found in a third study among subjects with dysplasia.⁵⁸

The possibility for a selenium effect earlier in the carcinogenesis also remains open. Any antioxidant effects^{2,3} of selenium, for instance, might occur early in carcinogenesis. In our interaction analyses of EAC, we found inverse associations

specifically for those subjects with a low intake of antioxidants. This finding could indicate an antioxidant mechanism of selenium for EAC. Other findings, however, did not match an antioxidant mechanism. We found an inverse association with EAC and GCA only in never smokers, whereas, in case of an antioxidant mechanism, one would expect these associations with selenium in the current smokers because smokers are exposed to oxidative stress,²⁹ and this oxidative stress could be counteracted by antioxidants.

The associations we observed between selenium and esophageal and gastric cancers may also be a result of other mechanisms. In fact, it has been suggested that selenium may act at a number of stages in carcinogenesis and by a number of different mechanisms (see introduction) that may operate simultaneously, or consecutively.³ The few *in vitro* and *vivo* studies that have been performed on selenium and esophageal carcinogenesis, show effects on different processes in early and late stages (eg, mutagenesis, development of precancerous lesions and cancer, cancer cell proliferation, and tumor growth).⁵⁹⁻⁶²

To learn more about the possible role of selenium in the etiology of ESCC, EAC, and GCA, the observations reported here need confirmation or refutation from other epidemiologic studies. Additionally, it would be interesting to study selenium in association with risk of Barrett's esophagus. A comparison of both associations may give more insight into the mechanism of action of selenium and the stage in the development of normal esophageal epithelium into EAC at which selenium exerts its effect.

Two strengths of our study are the size and the division of esophageal cancer into 2 main histologic subtypes: ESCC and EAC. This division enables the identification of possible differences in the etiology of these cancers. Thirdly, we are the first to investigate selenium in relation to risk of ESCC, EAC, and GCA in a Western population. Most previous studies were performed in Asia. A fourth strength is the prospective character of our study, which prevents bias by reverse causation, a concern in case-control studies. We investigated whether undiagnosed cancer at baseline may have influenced our results, by excluding cases diagnosed in the first 2 years of follow-up, but the results proved robust to this exclusion. Finally, the observation that our results hardly changed after adjustment for confounders, makes residual confounding unlikely.

Our study has some limitations that should be described. Toenail selenium levels were measured only once in our cohort. In the Nurses' Health Study, a correlation coefficient of 0.48 was found between toenail selenium levels for specimens taken 6 years apart.⁵³ This moderate reliability may have resulted in some misclassification of exposure. This misclassification is nondifferential and thus may have attenuated the association. Another limitation to our study was the relatively low number of female EAC and GCA cases, which prevented a more detailed investigation of the association with selenium by sex. As a final point, many statistical tests were performed, which increases the chance of false positive results.

In conclusion, this prospective study supports an inverse association between toenail selenium levels and risk of ESCC and GCA and suggests an inverse association with risk of EAC in subgroups (women, never smokers, and low antioxidant consumers).

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8

Vegetables and fruits consumption and risk of esophageal and gastric cancer subtypes in the Netherlands Cohort Study

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ABSTRACT

Prospective epidemiologic data on vegetables and fruits consumption and risk of subtypes of esophageal and gastric cancer are sparse. We studied the association between vegetables and fruits consumption and risk of esophageal squamous cell carcinoma (ESCC), esophageal adenocarcinoma (EAC), gastric cardia adenocarcinoma (GCA), and gastric noncardia adenocarcinoma (GNCA) in the Netherlands Cohort Study. In 1986, 120852 Dutch men and women aged 55-69 filled out a questionnaire on diet and other cancer risk factors. After 16.3 years of follow-up, 101 ESCC, 144 EAC, 156 GCA, 460 GNCA cases and 4035 subcohort members were available for case-cohort analysis using Cox proportional hazards models. Multivariable adjusted incidence rate ratios (RR) were generally below unity. Significant inverse associations were observed for raw vegetables and EAC risk [RR per 25g/day: 0.81, 95% confidence interval (CI) 0.68-0.98], and Brassica vegetables and GCA risk (RR per 25g/day: 0.72, 95% CI 0.54-0.95). Citrus fruits were inversely associated with EAC and GCA risk (RRs for highest vs. lowest intake: 0.55, 95% CI 0.31-0.98 and 0.38, 95% CI 0.21-0.69, respectively). Specifically for current smokers, vegetables and fruits intake was inversely associated with ESCC and EAC risk. Consumption of vegetables and fruits may protect against subtypes of esophageal and gastric cancer.

INTRODUCTION

Vegetables and fruits have been of interest for their possible favorable influence on risk of cancer and other diseases for some decades. These foods contain many substances that are potentially anticarcinogenic, such as vitamins, carotenoids, and flavonoids. Possible mechanisms of action include modulation of DNA methylation, protection from and repair of DNA damage, induction of detoxifying phase II enzymes, and promotion of apoptosis.^{1,2} A recent expert report concluded that consumption of vegetables and fruits probably protects against development of esophageal and gastric cancer.² Unfortunately, in this report, no distinction was made between the main subtypes of these cancers: esophageal squamous cell carcinoma (ESCC), esophageal adenocarcinoma (EAC), gastric cardia adenocarcinoma (GCA), and gastric noncardia adenocarcinoma (GNCA). It is interesting and important to look into subtypes of these cancers, as the evidence is growing that there are differences in risk factors between these cancers.³⁻⁵ Case-control studies on vegetables or fruits consumption and ESCC,⁶⁻⁹ EAC,^{7,8,10,11} GCA,^{7,8,12} or GNCA^{7,12} mostly reported inverse associations. However, case-control studies are vulnerable for biases because of their retrospective nature. This can specifically be a problem when investigating diet and gastrointestinal cancer.¹³ Cohort studies are less vulnerable for biases. Cohort studies that reported on ESCC have mostly found inverse associations,¹⁴⁻¹⁶ but some cohorts found no association with vegetables^{16,17} or fruits¹⁷ consumption. For EAC,^{14,18} GCA,^{16,18-21} and GNCA^{16,18-21} mainly null associations have been observed, except for two studies that found inverse associations for fruits consumption and GCA,¹⁶ and GNCA²⁰.

Using 3.3²² and 6.3¹³ year follow-up data of the Netherlands Cohort Study on diet and cancer (NLCS), we have previously reported on the associations between vegetables and fruits consumption and risk of total gastric cancer.¹³ Only for Allium vegetables, separate results for GCA and GNCA were presented.²²

The aim of this study was to prospectively investigate the role of vegetables and fruits consumption in the development of ESCC, EAC, GCA, and GNCA. Also, we aimed to study several groups of vegetables and fruits and individual vegetable and fruit items separately, as few studies have performed such comprehensive analyses. For this study, we used 16.3-year follow-up data from the NLCS.

MATERIALS AND METHODS

Study design and participants

This study was conducted within the prospective Netherlands Cohort Study, which started in September 1986 with the inclusion of 58279 men and 62573 women aged 55-69 years, who were randomly sampled from Dutch municipal registries.²³ At

baseline, all subjects completed a self-administered questionnaire on dietary and lifestyle habits and other cancer risk factors.

For reasons of efficiency, the case-cohort approach was used for data processing and analysis.^{24,25} Cases were derived from the entire cohort, and the number of person-years at risk for the entire cohort was estimated from a subcohort of 5000, who were randomly sampled from the total cohort at baseline. The subcohort has been followed up by contacting the participants and using data from municipal population registries.²³ Person-years at risk were calculated from the start of the study until esophageal or gastric cancer diagnosis, death, emigration, loss to follow-up or end of follow-up, whichever occurred first. Only one male subcohort member was lost to follow-up after 16.3 years.

Follow-up for cancer incidence was performed by record linkage²⁶ to the Netherlands Cancer Registry (NCR) and PALGA, the nationwide network and registry of histopathology and cytopathology in the Netherlands.²⁷ Data of 16.3 years of follow-up (baseline to December 31, 2002) were used. The completeness of cancer follow-up is $\geq 96\%$.²⁸ The following numbers of incident, microscopically confirmed, primary carcinomas were identified: 130 ESCC²⁹ (ICD-O-3 C15), 181 EAC²⁹ (C15), 206 GCA (C16.0) and 594 GNCA (C16.1-C16.9) (Figure 8.1). The group of GNCA included cancers with a lesion with overlapping subsites of the stomach (C16.8, $n=160$) and some gastric, not otherwise specified cancers (C16.9, $n=75$), raising the possibility that some cardia cancers might be included in the non-cardia category. However, as we found risk estimates to be similar in separate analyses of gastric cancers of specified sites (C16.1-C16.5) and other gastric cancers (C16.6-C16.9) (data not shown), we combined the groups in the analysis.

We excluded subjects who reported having prevalent cancer other than skin cancer at baseline. Also excluded were cases and subcohort members with incomplete or inconsistent dietary data.³⁰ Further details on the design of the study and methods of follow-up have been published previously.^{23,26} The Medical Ethics Committee of Maastricht University has approved the study.

Questionnaire data

The self-administered questionnaire included a 150-item food frequency questionnaire (FFQ). The FFQ concentrated on the habitual consumption of foods and beverages during the year preceding the start of the study.

With regard to vegetables consumption, subjects were asked to report their frequency of consumption of a number of vegetables (see Table 8.2), both in summer and in winter. They could choose one of six categories, ranging from “never or less than 1x per month” to “3-7x per week”. Subjects were asked about usual serving sizes only for string beans and cooked endive; the mean of these values served as an indicator to derive the serving sizes of all cooked vegetables, according to a vegetable-specific algorithm based on results of a pilot study. For onions and tomatoes,

participants were asked to report their consumption in number per week; for sweet peppers per month, and for mushrooms the number of 250 g boxes per month. Subjects with inconsistent vegetable data were excluded from analysis.

With regard to fruits consumption, subjects were asked to report the frequency and amount of consumption of a number of fruits (listed in Table 8.2). Categories ranged from “never or less than 1x per month” to “6-7x per week”. Using household units, these frequencies and amounts have been converted to consumption in grams/day.

The choice of items in the questionnaire was such that it covered almost all vegetables and fruits eaten regularly, with the exception of chicory, red cabbage, and cucumber. Furthermore, in an open-ended question, participants could fill in other foods they consumed regularly as well as the frequency and amount consumed on each occasion.

Questionnaire data were key-entered and processed in a standardized manner, blinded with respect to case/subcohort status to minimize observer bias in the coding and interpretation of the data.

Figure 8.1 Flow diagram of subcohort members and cases on whom the analyses were based. *PALGA: nationwide network and registry of histo- and cytopathology in the Netherlands, ESCC: esophageal squamous cell carcinoma, EAC: esophageal adenocarcinoma, GCA: gastric cardia adenocarcinoma, GNCA: gastric noncardia adenocarcinoma.

The FFQ was validated against a 9-day diet record.³⁰ The Spearman correlation coefficients were 0.38 for total vegetables consumption and 0.60 for total fruits consumption. On average, vegetables consumption appeared to be slightly overestimated and fruits consumption to be underestimated by the FFQ as compared with the diet records.

Statistical analysis

We checked for possible influence of preclinical cancer at baseline on the consumption of vegetables and fruits.¹³ This was done by comparing the mean consumption of vegetables and fruits for cases that were diagnosed during the first 2 years of follow-up with the mean consumption for cases diagnosed later in follow-up. An independent samples t-test was used to test for statistical significance of differences, after a square-root transformation was applied to normalize the distribution of the vegetables and fruits variables. Vegetables consumption was statistically significantly lower among early vs. late GCA (difference 43 g/day, $P=0.04$) and GNCA (difference 22 g/day, $P=0.02$) cases, while fruits consumption was lower among early vs. late ESCC cases (difference 74 g/day, $P=0.01$). Based on these observations, we decided to exclude the first 2 years of follow-up from all analyses, to prevent bias caused by reversed causation due to preclinical cancer.

Analyses were performed for total vegetables consumption, cooked and raw vegetables, several vegetable subgroups, and the most frequently consumed individual vegetables. Furthermore, we analyzed total fruits consumption, citrus fruits and the most frequently consumed individual fruits. The composition of each vegetable and fruit group can be found in the Appendix. For categorical analyses, vegetables and fruits consumption levels were categorized into quintiles according to the distribution in the subcohort. For continuous analyses, an increment of 25 g/day was chosen.

The following confounders were included in all models because they changed the incidence rate ratios (RR) by more than 5%: age (years), sex, cigarette smoking (current yes/no, number of cigarettes smoked daily, and number of smoking years), and consumption of alcohol (g/day), red meat (g/day), and fish (g/day). The following variables were considered potential confounders but were not included in the models because they did not change the RRs by more than 5%: consumption of tea, consumption of meat products, non-occupational physical activity, body mass index (BMI), highest educational level, family history of esophageal or gastric cancer, long-term use of non-steroidal anti-inflammatory drugs or aspirin, or lower esophageal sphincter (LES) relaxing medication.^{31,32} Analyses of vegetables were adjusted for fruits consumption and vice versa. For exact model specifications, see the table footnotes.

Cox proportional hazards models were used to estimate age- and sex-adjusted and multivariable adjusted RRs and corresponding 95% confidence intervals (CI).³³ Standard errors were estimated using a robust covariance matrix estimator to account for increased variance due to sampling from the cohort.²⁴ The proportional hazards

assumption was tested using the scaled Schoenfeld residuals.³⁴ Tests for dose-response trends were assessed by fitting ordinal exposure variables as continuous terms.

Because of the limited numbers of cases, specifically female cases, all analyses were carried out for both sexes combined. Nevertheless, analyses stratified by sex were performed for total vegetables and fruits consumption using continuous variables. We also investigated possible interaction between vegetables and fruits consumption and cigarette smoking status (current, former, never) by performing stratified analyses. P-values for interaction were assessed by including a cross-product term in the models.

All analyses were done using Stata 9.2 statistical software package (StataCorp, College Station, Texas, USA). The significance level α was set at 0.05. All reported p-values are two-sided.

RESULTS

Characteristics of the study population

Baseline characteristics of the subcohort and case groups are presented in Table 8.1. The most striking difference is the high percentage of men among EAC (78%) and GCA cases (85%), compared with the subcohort (49% men). Furthermore, cases were more likely current cigarette smokers than subcohort members and among ever smokers, cases smoked longer and more cigarettes than did subcohort members. Ethanol intake was also higher in all case groups than in the subcohort, particularly among ESCC cases. EAC cases had a higher BMI than the subcohort.

Baseline vegetables and fruits consumption

Table 8.2 presents baseline intakes of the individual vegetables and fruits that were listed in the questionnaire. Because the vegetables and fruits had a right-skewed distribution, we present medians and interquartile ranges. The vegetables and fruits are ranked by increasing percentage of nonusers in the subcohort. The median daily consumption of the subcohort was 179 grams of vegetables and 157 grams of fruits. The most frequently eaten vegetables in our population were string beans, cauliflower, and lettuce. When we look at the median daily consumption among users, the vegetables eaten in the largest amounts were tomatoes, onions, and string beans. The fruits eaten in the largest amounts were apples and pears, and oranges.

Table 8.1 Characteristics of cases and subcohort members in the Netherlands cohort study on diet and cancer (NLCS), 1986-2002.

Characteristic	Subcohort		Cases		
	(n=4035) ^a		ESCC (n=101) ^a	EAC (n=144) ^a	GNCA (n=460) ^a
Age at baseline (years)	61.3 (4.2)		62.6 (4.1)	61.4 (4.1)	62.5 (4.1)
Men	49%		54%	78%	66%
Level of education:					
primary	28%		34%	26%	38%
lower vocational	22%		20%	25%	24%
secondary and medium vocational	36%		34%	32%	29%
university and higher vocational	14%		12%	16%	9%
Cigarette smoking status:					
never smoker	37%		23%	19%	24%
former smoker	36%		30%	49%	40%
current smoker	27%		48%	32%	36%
Ever cigarette smokers:					
frequency of cigarette smoking (n/day)	15.2 (10.3)		18.1 (9.9)	20.0 (12.8)	16.2 (10.3)
duration of cigarette smoking (years)	31.5 (12.3)		36.1 (11.0)	33.8 (10.8)	34.5 (12.0)
Ethanol intake (g/day)	10.2 (14.2)		23.1 (28.4)	15.1 (18.0)	11.9 (15.8)
Body mass index (kg/m ²)	25.0 (3.1)		24.3 (3.6)	26.3 (3.4)	25.0 (3.2)
Non-occupational physical activity (min/day)	73.1 (60.6)		69.4 (65.7)	75.1 (57.1)	78.5 (69.6)
Family history of esophageal or gastric cancer	7%		10%	12%	13%
Use of NSAIDs ^b	6%		7%	8%	5%
Use of LES relaxing medication ^b	14%		16%	19%	15%

EAC, esophageal adenocarcinoma; ESCC, esophageal squamous cell carcinoma; GCA, gastric cardia adenocarcinoma; GNCA, gastric non-cardia adenocarcinoma; LES, lower esophageal sphincter; NSAIDs, non-steroidal anti-inflammatory drugs. Values are given as mean (SD); for categorical variables percentages are presented.

^a The number of subcohort members or cases used after exclusion of prevalent cancer cases, first two years of follow-up, subjects with inconsistent or incomplete questionnaire data, and subjects with missing data on alcohol consumption or cigarette smoking (status, frequency and duration); ^b longer than six months.

Table 8.2 Vegetables and fruit consumption of the subcohort members in the Netherlands Cohort Study on diet and cancer (NLCS), 1986-2002.

Food group or food item	Nonusers %	Users: daily intake (g)	
		median	(P25-P75)
Total vegetables consumption (n=3827)^a	0	179	(137-229)
String/French beans	2	17	(10-25)
Cauliflower	7	13	(8-20)
Lettuce	9	7	(4-11)
Carrots, cooked	12	8	(4-13)
Endive, cooked	14	12	(7-19)
Brussels sprouts	15	8	(5-12)
Sauerkraut	16	6	(3-9)
Tomatoes	18	24	(14-33)
Onion	20	22	(11-33)
Spinach	20	10	(6-16)
Beetroot	25	9	(5-14)
Kale	25	4	(2-5)
Cabbage	29	8	(4-13)
Leek	32	10	(5-17)
Dried pulses ^b	39	11	(4-17)
Mushrooms	39	4	(4-9)
Broad beans	43	6	(3-11)
Sweet peppers	47	4	(3-6)
Endive, raw	56	4	(2-7)
Rhubarb	59	3	(1-6)
Carrots, raw	67	5	(2-10)
Gherkins	70	3	(1-6)
Vegetable juices ^b	90	12	(5-25)
Total fruit consumption (n=4035)^c	1	157	(96-236)
Apples and pears	13	80	(45-116)
Strawberries	14	7	(4-11)
Oranges and fresh orange juice	16	42	(15-83)
Grapes	37	3	(1-8)
Mandarins	41	4	(2-8)
Bananas	47	11	(4-19)
Processed fruit juices ^b	51	23	(12-63)
Grapefruits and fresh grapefruit juice	70	16	(7-40)
Raisins and other dried fruits	75	1	(1-3)

^a The number of subcohort members used after exclusion of prevalent cancer cases, first two years of follow-up, subjects with inconsistent or incomplete questionnaire data, or inconsistent vegetable data, and subjects with missing data on alcohol consumption or cigarette smoking (status, frequency and duration); ^b Dried pulses and vegetable juices are not included in total vegetables consumption. Processed fruit juices are not included in total fruits consumption; ^c The number of subcohort members used after exclusion of prevalent cancer cases, first two years of follow-up, subjects with inconsistent or incomplete questionnaire data, and subjects with missing data on alcohol consumption or cigarette smoking (status, frequency and duration).

Main analyses

Results of the multivariable Cox regression analyses on total vegetables and fruits and subgroups of vegetables and fruits are shown in Table 8.3. The results from the age- and sex-adjusted analyses are presented in Supplementary Table 8.1, due to limited space. These results were comparable with the multivariable adjusted results.

For total vegetables consumption we observed that subjects in quintiles 2 to 5 had lower risks of esophageal and gastric cancers, when compared with quintile 1. The clearest inverse association was observed for EAC (RR for Q5 vs. Q1: 0.59, 95% CI 0.33-1.06), while the association was weakest for GNCA (RR for Q5 vs. Q1: 0.90, 95% CI 0.64-1.26). RRs or trends were not statistically significant for any tumor type.

For specific groups of vegetables, we can see that the majority of the RRs observed were below unity. These inverse associations, however, mostly did not reach statistical significance. No clear associations were observed for cooked vegetables, while for raw vegetables we found a significant inverse trend ($P=0.05$) for EAC risk (RR per 25 g/day increment: 0.80, 95% CI 0.68-0.98). Decreased risks associated with raw vegetables were also observed for ESCC and GCA, but these were not statistically significant. A quite strong, but not statistically significant, inverse association was observed between consumption of legumes and pulses and ESCC risk, while for GNCA this association was weaker, and no apparent associations were present for EAC and GCA. Consumption of Brassica vegetables was associated with a statistically significantly decreased risk of GCA (RR per 25 g/day: 0.72, 95% CI 0.54-0.95, p -trend=0.01), and slightly decreased risk of EAC (RR per 25 g/day: 0.85, 95% CI 0.49-1.48), while Brassicas were not clearly associated with risk of ESCC or GNCA. No clear associations were seen for consumption of Allium vegetables or cooked leafy vegetables and any tumor type, while for raw leafy vegetables inverse associations were seen for ESCC and EAC.

Also shown in Table 8.3 are RRs for consumption of total fruits and citrus fruits. Total fruits consumption was nonsignificantly inversely associated with ESCC risk (RR for Q5 vs. Q1: 0.62, 95% CI 0.32-1.22), while RRs only slightly below unity were seen for EAC, GCA, and GNCA. Citrus fruits were quite consistently associated with a decreased risk of all tumors: for ESCC, the RR for Q5 vs. Q1 was 0.54 (95% CI 0.27-1.07), for EAC this RR was 0.55 (95% CI 0.31-0.98), for GCA 0.38 (95% CI 0.21-0.69), and finally for GNCA this RR was 0.80 (95% CI, 0.56-1.15).

In Table 8.4, we present multivariable adjusted results for the individual vegetable and fruit items that were most frequently consumed in our cohort. Again, the age- and sex-adjusted results can be found in a supplement (i.e. Supplementary Table 8.2). None of the individual vegetable or fruit items was statistically significantly associated with risk of ESCC or EAC. For GCA, we observed inverse associations for cauliflower (RR per 25 g/day increment: 0.58, 95% CI 0.35-0.96), and oranges and fresh orange juices (RR per 25 g/day: 0.86, 95% CI 0.77-0.95). Positive associations were observed for tomato consumption and GNCA risk (RR per 25 g/day: 1.13, 95% CI 1.00-1.28), for spinach consumption and GCA risk (RR per 25 g/day: 1.77, 95% CI 1.04-3.02), and for apples and pears consumption and GCA risk (RR per 25 g/day: 1.05, 95% CI 1.00-1.09).

Table 8.3 Multivariable adjusted* associations between vegetables and fruit consumption and risk of esophageal and gastric cancer subtypes; Netherlands Cohort Study on diet and cancer (NLCS) 1986-2002^a

	Sub-cohort person years at risk	Esophageal squamous cell carcinoma		Esophageal adenocarcinoma		Gastric cardia adenocarcinoma		Gastric noncardia adenocarcinoma						
		cases (n) ^b	RR (95% CI)	cases (n) ^b	RR (95% CI)	cases (n) ^b	RR (95% CI)	cases (n) ^b	RR (95% CI)					
Total vegetables ^c														
Q1	104	9073	31	1	reference	35	1	reference	43	1	reference	111	1	reference
Q2	146	9829	22	0.74 (0.38-1.43)		30	0.71 (0.42-1.19)		26	0.63 (0.37-1.06)		86	0.79 (0.58-1.08)	
Q3	181	9662	17	0.56 (0.28-1.13)		25	0.61 (0.36-1.06)		27	0.64 (0.37-1.09)		91	0.88 (0.65-1.20)	
Q4	222	9835	12	1.36 (0.76-2.44)		21	0.83 (0.50-1.38)		27	0.87 (0.53-1.45)		79	0.80 (0.58-1.10)	
Q5	297	9823	14	0.61 (0.29-1.32)		26	0.59 (0.33-1.06)		25	0.87 (0.50-1.52)		76	0.90 (0.64-1.26)	
				p-trend ^d 0.67			p-trend 0.18			p-trend 0.93			p-trend 0.65	
continuous, per 25 g/day increment		48223	96	0.96 (0.89-1.04)		137	0.95 (0.89-1.02)		148	1.00 (0.94-1.07)		443	0.98 (0.94-1.02)	
Cooked vegetables ^e														
Q1	80	9495	22	1	reference	36	1	reference	33	1	reference	99	1	reference
Q2	115	9841	14	0.69 (0.34-1.38)		28	0.78 (0.47-1.30)		25	0.74 (0.43-1.26)		90	0.90 (0.66-1.22)	
Q3	144	10269	17	0.75 (0.38-1.45)		21	0.56 (0.32-0.97)		25	0.71 (0.41-1.22)		88	0.83 (0.61-1.13)	
Q4	177	10012	24	1.26 (0.69-2.29)		21	0.57 (0.32-1.02)		36	1.04 (0.64-1.70)		93	0.93 (0.68-1.27)	
Q5	239	8606	19	0.95 (0.45-2.02)		31	1.01 (0.59-1.73)		29	0.93 (0.53-1.63)		73	0.79 (0.56-1.13)	
				p-trend 0.62			p-trend 0.93			p-trend 0.76			p-trend 0.28	
continuous, per 25 g/day increment		48223	96	0.97 (0.89-1.06)		137	1.00 (0.91-1.09)		148	1.01 (0.93-1.09)		443	0.96 (0.92-1.01)	
Raw vegetables ^e														
Q1	8	9426	26	1	reference	31	1	reference	38	1	reference	95	1	reference
Q2	22	9550	17	0.72 (0.37-1.39)		37	1.22 (0.74-2.00)		29	0.79 (0.48-1.31)		79	0.87 (0.63-1.19)	
Q3	34	9805	19	0.84 (0.45-1.56)		24	0.84 (0.48-1.47)		24	0.74 (0.43-1.26)		97	1.15 (0.84-1.57)	
Q4	48	9968	17	0.70 (0.37-1.33)		30	1.00 (0.57-1.74)		29	0.86 (0.52-1.44)		88	1.05 (0.76-1.45)	
Q5	77	9473	17	0.70 (0.35-1.41)		15	0.53 (0.27-1.05)		28	0.93 (0.53-1.61)		84	1.16 (0.83-1.62)	
				p-trend 0.35			p-trend 0.05			p-trend 0.94			p-trend 0.22	
continuous, per 25 g/day increment		48223	96	0.94 (0.73-1.19)		137	0.81 (0.68-0.98)		148	0.99 (0.85-1.16)		443	1.04 (0.95-1.14)	
Legumes and pulses ^e														
Q1	11	9613	26	1	reference	26	1	reference	34	1	reference	98	1	reference
Q2	19	9595	13	0.56 (0.28-1.11)		23	0.89 (0.49-1.61)		28	0.80 (0.47-1.35)		85	0.92 (0.67-1.26)	
Q3	28	10408	25	0.88 (0.49-1.58)		29	0.98 (0.55-1.74)		22	0.51 (0.29-0.90)		92	0.83 (0.61-1.14) ^f	
Q4	39	9903	17	0.65 (0.33-1.31)		23	0.83 (0.45-1.54)		40	0.99 (0.60-1.62)		89	0.87 (0.63-1.20)	
Q5	62	8704	15	0.57 (0.26-1.27)		36	1.42 (0.81-2.51)		24	0.60 (0.34-1.06)		79	0.83 (0.59-1.18)	
				p-trend 0.26			p-trend 0.15			p-trend 0.28			p-trend 0.36	
continuous, per 25 g/day increment		48223	96	0.81 (0.58-1.13)		137	1.12 (0.93-1.34)		148	0.96 (0.76-1.20)		443	0.90 (0.78-1.03)	

	Sub-cohort	Esophageal squamous cell carcinoma		Esophageal adenocarcinoma		Gastric cardia adenocarcinoma		Gastric noncardia adenocarcinoma						
		person years at risk	cases (n) ^b	RR (95% CI)	cases (n) ^b	RR (95% CI)	cases (n) ^b	RR (95% CI)	cases (n) ^b	RR (95% CI)				
Brassica vegetables ^e														
Q1	1	9569	19	1	reference	35	1	reference	38	1	reference			
Q2	21	10064	22	1.20 (0.63-2.28)	26	0.77 (0.45-1.31)	26	0.77 (0.45-1.31)	35	0.87 (0.54-1.40)	74	0.71 (0.52-0.98)		
Q3	29	9804	19	1.16 (0.60-2.24)	27	0.78 (0.46-1.33)	27	0.78 (0.46-1.33)	25	0.56 (0.34-0.95)	83	0.81 (0.59-1.11)		
Q4	39	9879	20	1.26 (0.63-2.52)	22	0.62 (0.34-1.12)	22	0.62 (0.34-1.12)	24	0.50 (0.29-0.86)	100	1.02 (0.75-1.40)		
Q5	59	8906	16	1.08 (0.47-2.49)	27	0.85 (0.49-1.48)	27	0.85 (0.49-1.48)	26	0.51 (0.28-0.92)	83	0.95 (0.66-1.35)		
				p-trend 0.86		p-trend 0.54		p-trend 0.54		p-trend 0.01		p-trend 0.62		
continuous, per 25 g/day increment		48223	96	0.99 (0.70-1.39)	137	1.06 (0.77-1.46) ^g	137	1.06 (0.77-1.46) ^g	148	0.72 (0.54-0.95)	443	1.05 (0.90-1.21)		
Allium vegetables ^e														
Q1	5	13046	25	1	reference	49	1	reference	37	1	reference	118	1	reference
Q2	16	6359	11	1.00 (0.48-2.10)	16	0.67 (0.37-1.20)	16	0.67 (0.37-1.20)	17	0.92 (0.50-1.68)	76	1.32 (0.96-1.83)		
Q3	25	9686	19	1.07 (0.56-2.02)	22	0.64 (0.38-1.10)	22	0.64 (0.38-1.10)	35	1.35 (0.83-2.19)	72	0.85 (0.62-1.18)		
Q4	38	9920	14	0.80 (0.41-1.58)	23	0.65 (0.38-1.11)	23	0.65 (0.38-1.11)	24	0.95 (0.55-1.65)	104	1.25 (0.93-1.68)		
Q5	62	9213	27	1.64 (0.88-3.08)	27	0.84 (0.50-1.43)	27	0.84 (0.50-1.43)	35	1.55 (0.94-2.56)	73	0.97 (0.69-1.35)		
				p-trend 0.18		p-trend 0.54		p-trend 0.54		p-trend 0.12		p-trend 0.95		
continuous, per 25 g/day increment		48223	96	1.15 (0.90-1.47) ^g	137	0.96 (0.77-1.20)	137	0.96 (0.77-1.20)	148	1.14 (0.97-1.35)	443	0.98 (0.87-1.11)		
Leafy vegetables, cooked ^e														
Q1	4	9505	21	1	reference	30	1	reference	29	1	reference	94	1	reference
Q2	12	10208	22	0.87 (0.45-1.68)	31	0.95 (0.56-1.60)	31	0.95 (0.56-1.60)	32	1.00 (0.59-1.69)	101	0.95 (0.70-1.29)		
Q3	19	9557	19	1.00 (0.53-1.89)	28	0.96 (0.56-1.65)	28	0.96 (0.56-1.65)	32	1.13 (0.66-1.94)	79	0.83 (0.60-1.14) ^g		
Q4	27	9803	21	1.07 (0.55-2.08)	24	0.76 (0.42-1.36)	24	0.76 (0.42-1.36)	26	0.93 (0.53-1.65)	89	0.92 (0.67-1.27) ^f		
Q5	42	9150	13	0.75 (0.35-1.60)	24	0.83 (0.47-1.46)	24	0.83 (0.47-1.46)	29	1.18 (0.66-2.09)	80	0.87 (0.61-1.23)		
				p-trend 0.66		p-trend 0.40		p-trend 0.40		p-trend 0.65		p-trend 0.45		
continuous, per 25 g/day increment		48223	96	0.94 (0.66-1.33)	137	0.89 (0.65-1.22)	137	0.89 (0.65-1.22)	148	1.29 (0.96-1.74)	443	0.92 (0.76-1.10)		
Leafy vegetables, raw ^e														
Q1	1	9254	24	1	reference	34	1	reference	32	1	reference	101	1	reference
Q2	4	10167	17	0.70 (0.36-1.35)	32	0.89 (0.54-1.47)	32	0.89 (0.54-1.47)	27	0.79 (0.46-1.35)	94	0.92 (0.67-1.25)		
Q3	7	9573	21	0.87 (0.46-1.67)	29	0.83 (0.49-1.41)	29	0.83 (0.49-1.41)	28	0.82 (0.48-1.39)	90	0.93 (0.68-1.28)		
Q4	12	9649	22	1.03 (0.56-1.92)	21	0.62 (0.35-1.09)	21	0.62 (0.35-1.09)	33	0.97 (0.57-1.63)	76	0.81 (0.58-1.13)		
Q5	22	9581	12	0.50 (0.23-1.10)	21	0.63 (0.35-1.13)	21	0.63 (0.35-1.13)	28	0.86 (0.48-1.54)	82	0.92 (0.65-1.30)		
				p-trend 0.17		p-trend 0.08		p-trend 0.08		p-trend 0.92		p-trend 0.59		
continuous, per 25 g/day increment		48223	96	0.83 (0.36-1.89) ^g	137	0.62 (0.33-1.15)	137	0.62 (0.33-1.15)	148	0.89 (0.54-1.47)	443	0.86 (0.62-1.18) ^f		

	categorical median (g/day)	Sub-cohort person years at risk	Esophageal squamous cell carcinoma		Esophageal adenocarcinoma		Gastric cardia adenocarcinoma		Gastric noncardia adenocarcinoma					
			cases (n) ^a	RR (95% CI)	cases (n) ^a	RR (95% CI)	cases (n) ^a	RR (95% CI)	cases (n) ^a	RR (95% CI)				
Total fruit ^h														
Q1	43	9508	33	1	reference	39	1	reference	46	1	reference			
Q2	107	10213	22	0.77 (0.43-1.38)		31	0.89 (0.54-1.47)		26	0.63 (0.38-1.04)	90	0.84 (0.62-1.13)		
Q3	155	10123	18	0.72 (0.39-1.33)		26	0.81 (0.48-1.37)		28	0.75 (0.46-1.21)	94	0.95 (0.70-1.28)		
Q4	215	10368	13	0.53 (0.27-1.04)		22	0.73 (0.41-1.29)		30	0.86 (0.53-1.40)	83	0.83 (0.61-1.13)		
Q5	326	10573	15	0.62 (0.32-1.22)		26	0.97 (0.57-1.67)		26	0.85 (0.50-1.42)	80	0.86 (0.62-1.18)		
				p-trend 0.11			p-trend 0.77			p-trend 0.82		p-trend 0.39		
continuous, per 25 g/day increment		50785	101	0.95 (0.90-1.01)		144	1.00 (0.96-1.05)		156	0.99 (0.95-1.03)	460	1.00 (0.97-1.02)		
Citrus fruits ^{ij}														
C1	0	5048	18	1	reference	30	1	reference	32	1	reference	61	1	reference
C2	8	11169	19	0.47 (0.24-0.95)		35	0.59 (0.35-1.00)		47	0.76 (0.47-1.22)	104	0.86 (0.61-1.21)		
C3	33	11494	27	0.72 (0.37-1.43)		24	0.46 (0.26-0.81)		30	0.54 (0.32-0.92)	100	0.89 (0.62-1.27)		
C4	77	11490	18	0.51 (0.25-1.04)		29	0.60 (0.35-1.05)		28	0.55 (0.32-0.94)	109	0.99 (0.70-1.40)		
C5	156	11583	19	0.54 (0.27-1.07)		26	0.55 (0.31-0.98)		19	0.38 (0.21-0.69)	86	0.80 (0.56-1.15)		
				p-trend 0.38			p-trend 0.37			p-trend 0.003		p-trend 0.46		
continuous, per 25 g/day increment		50785	101	1.01 (0.92-1.10)		144	0.97 (0.90-1.04)		156	0.88 (0.81-0.97)	460	0.99 (0.95-1.03)		

C, category; CI, confidence interval; RR, Incidence Rate Ratio; Q, quintile. * Adjusted for age (years), sex, cigarette smoking [(current smoking (yes/no), frequency (number of cigarettes per day), duration (number of years)], alcohol consumption (g ethanol/day), consumption of red meat (g/day), consumption of fish (g/day)].^a The first two years of the follow-up were excluded from all analyses, see text; ^b The number of cases in the vegetable analyses is lower than in the fruit analyses, due to the exclusion of subjects with inconsistent vegetable data; ^c Additionally adjusted for total fruit intake; ^d Tests for dose-response trends were assessed by fitting ordinal exposure variables as continuous terms in the Cox proportional hazards model; ^e Additionally adjusted for total fruit intake and all other vegetables; ^f The proportional hazards assumption was violated for the exposure variable in this analysis. The interaction between the exposure variable and time was statistically significant; ^g The proportional hazards assumption was violated for the exposure variable in this analysis. The interaction between the exposure variable and time was not statistically significant; ^h Additionally adjusted for total vegetable intake; ⁱ Additionally adjusted for total vegetable intake and all other fruits; ^j Due to a large number of nonusers of citrus fruits, categories instead of quintiles were used in analyses: C1 are non-users, C2 to C5 are users, divided into quartiles.

Table 8.4 Multivariable adjusted* associations between consumption of individual vegetable and fruit items and risk of esophageal and gastric cancer subtypes; Netherlands Cohort Study on diet and cancer (NLCS) 1986-2002^a

Vegetable item ^d	Subcohort		Esophageal squamous cell carcinoma		Esophageal adenocarcinoma		Gastric cardia adenocarcinoma		Gastric noncardia adenocarcinoma	
	person years at risk		cases (n) ^b	RR ^c (95% CI)	cases (n) ^b	RR ^c (95% CI)	cases (n) ^b	RR ^c (95% CI)	cases (n) ^b	RR ^c (95% CI)
String/French beans	48223		96	0.80 (0.50-1.28)	137	1.13 (0.85-1.50)	148	0.91 (0.64-1.31) ^e	443	0.86 (0.68-1.08)
Cauliflower	48223		96	0.94 (0.52-1.70)	137	0.88 (0.51-1.52)	148	0.58 (0.35-0.96)	443	1.04 (0.77-1.39)
Lettuce	48223		96	0.72 (0.28-1.85)	137	0.51 (0.22-1.17)	148	1.12 (0.58-2.17)	443	0.87 (0.58-1.31) ^f
Carrots, cooked	48223		96	1.12 (0.57-2.22)	137	0.85 (0.44-1.64)	148	0.72 (0.40-1.31) ^f	443	0.79 (0.55-1.13)
Endive, cooked	48223		96	0.90 (0.52-1.57)	137	0.92 (0.55-1.54)	148	1.03 (0.70-1.51)	443	0.80 (0.60-1.07)
Brussels sprouts	48223		96	1.29 (0.71-2.34)	137	1.37 (0.69-2.71)	148	1.14 (0.55-2.36)	443	1.24 (0.85-1.80)
Sauerkraut	48223		96	0.56 (0.16-2.00)	137	1.45 (0.58-3.68)	148	0.86 (0.36-2.07)	443	1.03 (0.63-1.69)
Tomatoes	48223		96	0.87 (0.62-1.22)	137	0.79 (0.61-1.01)	148	0.93 (0.71-1.23)	443	1.13 (1.00-1.28)
Onion	48223		96	1.10 (0.79-1.53)	137	0.84 (0.65-1.09)	148	1.21 (0.95-1.53)	443	0.91 (0.78-1.06)
Spinach	48223		96	1.02 (0.51-2.05) ^e	137	0.80 (0.44-1.45)	148	1.77 (1.04-3.02)	443	1.02 (0.73-1.44) ^e
Beetroot	48223		96	0.90 (0.41-1.96)	137	1.09 (0.60-2.00)	148	1.49 (0.84-2.63)	443	1.13 (0.74-1.75)
Kale	48223		96	0.55 (0.09-3.45)	137	1.12 (0.32-3.92) ^e	148	1.17 (0.34-4.02)	443	1.69 (0.76-3.75)
Fruit item^g										
Apples and pears	50785		101	0.94 (0.87-1.03)	144	1.04 (0.99-1.10)	156	1.05 (1.00-1.09)	460	1.00 (0.96-1.03)
Strawberries	50785		101	0.83 (0.40-1.73)	144	0.86 (0.47-1.57)	156	1.06 (0.60-1.85) ^f	460	0.96 (0.69-1.32)
Oranges and fresh orange juice	50785		101	1.01 (0.91-1.13)	144	0.95 (0.87-1.04)	156	0.86 (0.77-0.95)	460	0.99 (0.95-1.04)

CI, confidence interval; RR, Incidence Rate Ratio. * Adjusted for age (years), sex, cigarette smoking [(current smoking (yes/no), frequency (number of cigarettes/day), duration (number of years)], alcohol consumption (g ethanol/day), consumption of red meat (g/day), consumption of fish (g/day)).^a The first two years of the follow-up were excluded from all analyses, see text.^b The number of cases in the vegetable analyses is lower than in the fruit analyses, due to the exclusion of subjects with inconsistent vegetable data.^c Continuous variables, RR per 25 g/day increments.^d Additionally adjusted for total fruit intake. The individual vegetable items were adjusted for all other vegetables consumed.^e The proportional hazards assumption was violated for the exposure variable in this analysis. The interaction between exposure variable and time was statistically significant.^f The proportional hazards assumption was violated for the exposure variable in this analysis. The interaction between the exposure variable and time was not statistically significant.^g Additionally adjusted for total vegetable intake. The individual fruit items were adjusted for all other fruits consumed.

Table 8.5 Multivariable adjusted* associations between consumption of individual vegetable and fruit items and risk of esophageal and gastric cancer subtypes, stratified by sex and cigarette smoking status; Netherlands Cohort Study on diet and cancer (NLCS) 1986-2002^a

	Subcohort		Esophageal squamous cell carcinoma		Esophageal adenocarcinoma		Gastric cardia adenocarcinoma		Gastric noncardia adenocarcinoma				
	person	years at risk	cases (n) ^b	RR	(95% CI)	cases (n) ^b	RR	(95% CI)	cases (n) ^b	RR	(95% CI)		
Total vegetables ^c , RRs per 25 g/day increments													
Sex													
Men	22621	54	0.90	(0.80-1.00)	106	0.99	(0.91-1.06)	125	0.99	(0.92-1.07)	294	0.96	(0.92-1.01)
Women	25601	42	1.03	(0.95-1.12)	31	0.86	(0.75-0.97)	23	1.04	(0.93-1.16)	149	1.01	(0.95-1.07)
			p-interaction ^d 0.04			p-interaction ^d 0.06			p-interaction ^d 0.53			p-interaction ^d 0.24	
Cigarette smoking status													
Never smoker	18728	22	1.08	(0.98-1.19)	26	0.97	(0.84-1.13)	24	1.11	(0.99-1.25)	105	1.01	(0.94-1.09)
Former smoker	17369	28	0.96	(0.83-1.11)	68	1.02	(0.93-1.11)	72	0.95	(0.86-1.05)	180	0.98	(0.92-1.04)
Current smoker	12126	46	0.90	(0.81-0.99)	43	0.85	(0.75-0.97)	52	0.99	(0.90-1.10)	158	0.96	(0.90-1.01)
			p-interaction ^d 0.03			p-interaction ^d 0.08			p-interaction ^d 0.12			p-interaction ^d 0.51	
Total fruit ^e , RRs per 25 g/day increments													
Sex													
Men	23722	55	0.91	(0.83-1.00)	112	1.00	(0.96-1.05)	132	0.96	(0.92-1.01)	304	0.99	(0.96-1.02)
Women	27062	46	0.98	(0.91-1.05)	32	0.98	(0.91-1.06)	24	1.08	(1.02-1.14)	156	1.00	(0.97-1.04)
			p-interaction ^d 0.24			p-interaction ^d 0.65			p-interaction ^d 0.003			p-interaction ^d 0.47	
Cigarette smoking status													
Never smoker	19774	23	1.01	(0.94-1.08)	28	0.99	(0.92-1.07)	27	1.02	(0.95-1.10)	110	0.99	(0.95-1.03)
Former smoker	18099	30	0.94	(0.85-1.03)	70	1.03	(0.97-1.08)	74	0.99	(0.95-1.04)	186	1.01	(0.97-1.04)
Current smoker	12912	48	0.91	(0.82-1.01)	46	0.93	(0.86-1.01)	55	0.95	(0.87-1.04)	164	0.98	(0.93-1.03)
			p-interaction ^d 0.25			p-interaction ^d 0.15			p-interaction ^d 0.49			p-interaction ^d 0.63	

CI, confidence interval; RR, Incidence Rate Ratio. * Adjusted for age (years), sex, cigarette smoking [(current smoking (yes/no), frequency (number of cigarettes per day), duration (number of years)], alcohol consumption (g ethanol/day), consumption of red meat (g/day), consumption of fish (g/day)).^a The first two years of the follow-up were excluded from all analyses, see text.^b The number of cases in the vegetable analyses is lower than in the fruit analyses, due to the exclusion of subjects with inconsistent vegetable data; ^c Additionally adjusted for total fruit intake; ^d p-values for interaction were calculated by including a cross-product term in the Cox proportional hazards model; ^e Additionally adjusted for total vegetable intake.

Interaction analyses

Results of analyses stratified by sex and smoking status are presented in Table 8.5 (multivariable adjusted) and Supplemental Table 8.3 (age- and sex-adjusted). Adjustment for confounding variables did not change the RRs.

Total vegetables consumption was associated with risk of ESCC only in men (RR per 25 g/day increment: 0.90, 95% CI 0.80-1.00, p -interaction=0.04), whereas for EAC an inverse association was observed in women only (RR per 25 g/day: 0.86, 95% CI 0.75-0.97, p -interaction=0.06). For GCA and GNCA, no clear sex differences were observed. In current cigarette smokers only, we found significant inverse associations between vegetables consumption and risk of ESCC (RR per 25 g/day: 0.90, 95% CI 0.81-0.99, p -interaction=0.03) and EAC (RR per 25 g/day: 0.85, 95% CI 0.75-0.97, p -interaction=0.08). We found no interaction between vegetables consumption and smoking for GCA and GNCA.

An inverse association between consumption of fruits and ESCC was present in women (RR per 25 g/day: 0.91, 95% CI 0.83-1.00), but the interaction with sex was not statistically significant (p -interaction=0.24). For women, a positive association between fruits consumption and GCA risk was observed (RR per 25 g/day: 1.08, 95% CI 1.02-1.14, p -interaction=0.003). No significant interactions between smoking status and fruits consumption were observed, although for ESCC, EAC, and GCA the risk estimates were further below one for current smokers than for never and former smokers.

DISCUSSION

In the Netherlands Cohort Study, we found mostly inverse associations between vegetables and fruits consumption and risk of esophageal and gastric cancer subtypes. Most RRs were not statistically significant. However, our extensive questions on specific vegetables and fruits allowed us to study these foods in more detail. We identified significant inverse associations for raw vegetables and EAC risk, Brassicas and GCA risk, and citrus fruits and EAC and GCA risk. Associations were generally stronger inverse in current smokers, but the interaction tests were not always statistically significant.

The current evidence from case-control and cohort studies does not clearly point to any specific group of vegetables that is responsible for the observed inverse associations. For ESCC, significant inverse associations have been observed with Cruciferous vegetables in one cohort study,¹⁵ and with cooked vegetables,³⁵ raw vegetables,^{7,36} tomatoes³⁷ and spinach³⁷ in case-control studies. The few studies on EAC found significant inverse associations with raw and cooked spinach¹⁴, dark green vegetables,⁷ dark yellow vegetables,^{7,38} and raw vegetables.⁷ Only one study found significant inverse associations with GCA; for dark green vegetables and beetroot.³⁹

Beetroot, carrots, Allium vegetables,³⁹ dark green, light green, and yellow vegetables⁴⁰ have shown significant inverse associations with GNCA in two studies. This diversity of vegetables for which significant inverse associations have been observed may have to do with the fact that vegetable consumption habits differ between continents, countries, and populations with respect to the amount and types eaten.

For fruit groups, the evidence is more consistent than for vegetable groups. Citrus fruits have been shown to be inversely associated with risk of ESCC,^{14,35,36} EAC,³⁸ GCA,¹⁸ and GNCA^{18,39} by a considerable number of studies. Some other fruits that showed significantly lower risks were Rosaceae for ESCC,¹⁴ fruit juices for GCA and GNCA,³⁹ and apples and pears for GNCA.³⁹

Our data suggest that for current smokers, decreased risks of EAC and ESCC associated with vegetables and fruits consumption were more apparent than for never and former smokers. This finding is consistent with three case-control studies,^{6,8,36} which found inverse associations between vegetables consumption and ESCC only⁶ or most strongly^{8,36} in smokers. Cohort studies found no interactions for ESCC,^{14,15} EAC,¹⁴ GCA,¹⁹ or GNCA.¹⁹ Smoking causes oxidative stress and DNA damage in the body.⁴¹ Substances in vegetables and fruits that have anticarcinogenic properties (e.g. vitamin C, carotenoids, flavonoids, folate) could counteract these effects. Smokers could therefore benefit most from consuming vegetables and fruits.

Case-control studies have generally found stronger and more often statistically significant associations compared with cohort studies. An explanation may be reversed causation. Individuals who are diagnosed with gastrointestinal cancer may already have experienced intestinal complaints quite some time before the diagnosis. This may have caused them to change their dietary habits, including consumption of vegetables and fruits, leading to biased measurement of the diet as it was before the onset of the disease.¹³ Asking about dietary habits long before the diagnosis will not solve this problem, as reporting past diet is influenced by current diet.⁴² Cohort studies have fewer problems with reversed causation thanks to their prospective nature. Additionally, we minimized the influence of reversed causation on our results by excluding early cases. On the other hand, measurement of vegetables and fruits in cohort studies is not perfect either. It is very difficult for persons to accurately report their intake, specifically of vegetables. This is reflected in the relatively low correlations in validation studies.⁴³ A consequence of these low correlations is attenuation of risk estimates. Thus, the true associations may have been stronger than we observed, strengthening our conclusion of protective effects of some vegetables and fruits on esophageal and gastric cancers. Besides the attenuation of RRs, another explanation for the nonsignificance of the results is the limited power. Even though one strength of the NLCS is its large size, the number of ESCC, EAC, and GCA cases is limited. The large size of the study, combined with a long follow-up did allow for separate analysis of ESCC vs. EAC and GCA vs. GNCA. A strength of our study was the ability to check for confounding by several important risk factors (see methods), and to make adjustments if necessary. One factor that we did not measure and may have confounded the

associations with GNCA in particular is infection with *Helicobacter pylori*, which is a risk factor for GNCA and may protect against GCA. We estimated that half of our study population might have been infected.^{44,45} It is not clear whether adjustment for *Helicobacter pylori* infection would have changed our results. One study that made this adjustment,⁴⁶ found no effect on the estimates. Two studies that looked into interaction with *Helicobacter pylori* found no evidence of this.^{18,46}

In conclusion, we observed significant inverse associations between some specific vegetables and fruits and risk of ESCC, EAC, GCA, and GNCA. Most other observed associations were inverse, but not statistically significant. Consumption of fruits and vegetables may specifically help protect smokers from developing ESCC, EAC, and GCA.

APPENDIX

Composition of vegetable and fruit groups

Food group	Composition
Total vegetables	Cooked vegetables plus raw vegetables
Cooked vegetables	Beetroot, broad beans, Brussels sprouts, cauliflower, cabbage (white/green), cooked carrots, cooked endive, kale, leek, mushrooms, onions, rhubarb, sauerkraut, spinach, string beans, sweet peppers, and other cooked vegetables originating from an open-ended question on frequently consumed items not listed in the questionnaire.
Raw vegetables	Gherkins, lettuce, raw carrots, raw endive, tomatoes, and other raw vegetables originating from an open ended question on frequently consumed items not listed in the questionnaire
Brassica vegetables	Brussels sprouts, cabbage (white/green), cauliflower, kale
Leafy vegetables, cooked	Cooked endive, spinach
Leafy vegetables, raw	Lettuce, raw endive
Legumes and pulses	Broad beans, dried pulses, string beans
Allium vegetables	Leek, onions
Total fruit	Apples/pears, bananas, grapefruits and fresh grapefruit juice, grapes, mandarins, oranges and fresh orange juice, raisins/other dried fruit, strawberries, and other fruits originating from an open ended question on frequently consumed items not listed in the questionnaire.
Citrus fruits	Fresh lemon juice, grapefruits and fresh grapefruit juice, mandarins, oranges and fresh orange juice

Supplemental Table 8.1 Age- and sex-adjusted associations between vegetables and fruit consumption and risk of esophageal and gastric cancer subtypes; Netherlands Cohort Study on diet and cancer (NLCS) 1986-2002^a.

	categorical median (g/day)	Sub-cohort person years at risk	Esophageal squamous cell carcinoma			Esophageal adenocarcinoma			Gastric cardia adenocarcinoma			Gastric noncardia adenocarcinoma		
			cases (n) ^b	RR	(95% CI)	cases (n) ^b	RR	(95% CI)	cases (n) ^b	RR	(95% CI)	cases (n) ^b	RR	(95% CI)
Total vegetables														
Q1	104	9073	31	1	reference	35	1	reference	43	1	reference	111	1	reference
Q2	146	9829	22	0.71	(0.38-1.35)	30	0.71	(0.42-1.19)	26	0.62	(0.37-1.05)	86	0.78	(0.57-1.06)
Q3	181	9662	17	0.54	(0.27-1.07)	25	0.62	(0.36-1.07)	27	0.62	(0.37-1.06)	91	0.87	(0.64-1.17)
Q4	222	9835	12	1.24	(0.71-2.17)	21	0.85	(0.52-1.41)	27	0.87	(0.54-1.42)	79	0.78	(0.57-1.07)
Q5	297	9823	14	0.69	(0.35-1.36)	26	0.66	(0.38-1.16)	25	0.88	(0.53-1.45)	76	0.88	(0.64-1.21)
				p-trend ^c	0.83		p-trend	0.31		p-trend	0.91		p-trend	0.53
continuous, per 25 g/day increment		48223	96	0.99	(0.92-1.06)	137	0.96	(0.90-1.03)	148	1.00	(0.94-1.07)	443	0.98	(0.94-1.01)
Cooked vegetables														
Q1	80	9495	22	1	reference	36	1	reference	33	1	reference	99	1	reference
Q2	115	9841	14	0.62	(0.31-1.23)	28	0.75	(0.45-1.25)	25	0.73	(0.43-1.25)	90	0.89	(0.65-1.20)
Q3	144	10269	17	0.73	(0.38-1.38)	21	0.54	(0.31-0.94)	25	0.71	(0.41-1.21)	88	0.84	(0.61-1.14)
Q4	177	10012	24	1.08	(0.60-1.95)	21	0.55	(0.32-0.95)	36	1.03	(0.63-1.68)	93	0.92	(0.68-1.25)
Q5	239	8606	19	0.97	(0.52-1.81)	31	0.93	(0.57-1.53)	29	0.95	(0.57-1.59)	73	0.82	(0.59-1.13)
				p-trend	0.56		p-trend	0.73		p-trend	0.69		p-trend	0.33
continuous, per 25 g/day increment		48223	96	1.00	(0.92-1.08)	137	0.99	(0.91-1.07)	148	1.01	(0.94-1.09)	443	0.97	(0.93-1.01)
Raw vegetables														
Q1	8	9426	26	1	reference	31	1	reference	38	1	reference	95	1	reference
Q2	22	9550	17	0.64	(0.34-1.18)	37	1.17	(0.72-1.92)	29	0.76	(0.46-1.25)	79	0.82	(0.59-1.12)
Q3	34	9805	19	0.72	(0.40-1.30)	24	0.82	(0.47-1.42)	24	0.70	(0.41-1.18)	97	1.05	(0.78-1.43)
Q4	48	9968	17	0.63	(0.34-1.17)	30	1.00	(0.60-1.69)	29	0.82	(0.50-1.35)	88	0.93	(0.68-1.27)
Q5	77	9473	17	0.69	(0.37-1.29)	15	0.55	(0.29-1.05)	28	0.88	(0.54-1.46)	84	0.99	(0.72-1.35)
				p-trend	0.33		p-trend	0.04		p-trend	0.82		p-trend	0.80
continuous, per 25 g/day increment		48223	96	0.93	(0.74-1.16)	137	0.83	(0.70-0.98)	148	0.98	(0.85-1.14)	443	1.00	(0.91-1.09)

categorical median (g/day)	Sub-cohort person years at risk	Esophageal squamous cell carcinoma			Esophageal adenocarcinoma			Gastric cardia adenocarcinoma			Gastric noncardia adenocarcinoma		
		cases (n) ^b	RR	(95% CI)	cases (n) ^b	RR	(95% CI)	cases (n) ^b	RR	(95% CI)	cases (n) ^b	RR	(95% CI)
Legumes and pulses													
11 Q1	9613	26	1	reference	26	1	reference	34	1	reference	98	1	reference
19 Q2	9595	13	0.53	(0.27-1.04)	23	0.86	(0.48-1.54)	28	0.79	(0.47-1.33)	85	0.90	(0.66-1.23)
28 Q3	10408	25	0.88	(0.50-1.56)	29	0.91	(0.53-1.58)	22	0.51	(0.30-0.90)	92	0.82	(0.61-1.12) ^d
39 Q4	9903	17	0.64	(0.34-1.21)	23	0.78	(0.43-1.38)	40	1.00	(0.62-1.61)	89	0.85	(0.63-1.16)
62 Q5	8704	15	0.63	(0.33-1.22)	36	1.32	(0.78-2.23)	24	0.65	(0.38-1.11)	79	0.84	(0.61-1.16)
				p-trend 0.30			p-trend 0.22			p-trend 0.35			p-trend 0.34
continuous, per 25 g/day increment	48223	96	0.90	(0.69-1.18)	137	1.09	(0.92-1.31)	148	0.97	(0.78-1.20)	443	0.91	(0.80-1.03)
Brassica vegetables													
11 Q1	9569	19	1	reference	35	1	reference	38	1	reference	103	1	reference
21 Q2	10064	22	1.11	(0.60-2.07)	26	0.72	(0.43-1.22)	35	0.90	(0.56-1.46)	74	0.69	(0.51-0.96)
29 Q3	9804	19	0.98	(0.51-1.86)	27	0.74	(0.44-1.24)	25	0.63	(0.37-1.05)	83	0.78	(0.57-1.06)
39 Q4	9879	20	1.04	(0.55-1.97)	22	0.59	(0.34-1.03)	24	0.59	(0.35-1.01)	100	0.94	(0.70-1.27)
59 Q5	8906	16	0.91	(0.46-1.79)	27	0.79	(0.47-1.32)	26	0.69	(0.41-1.16)	83	0.85	(0.62-1.15)
				p-trend 0.71			p-trend 0.40			p-trend 0.10			p-trend 0.88
continuous, per 25 g/day increment	48223	96	0.93	(0.71-1.23)	137	1.00	(0.75-1.33) [§]	148	0.84	(0.65-1.08)	443	1.00	(0.88-1.13)
Allium vegetables													
5 Q1	13046	25	1	reference	49	1	reference	37	1	reference	118	1	reference
16 Q2	6359	11	0.92	(0.45-1.89)	16	0.66	(0.37-1.17)	17	0.92	(0.51-1.66)	76	1.34	(0.98-1.83)
25 Q3	9686	19	1.04	(0.57-1.91)	22	0.61	(0.37-1.03)	35	1.30	(0.81-2.09)	72	0.84	(0.62-1.15)
38 Q4	9920	14	0.78	(0.40-1.51)	23	0.66	(0.40-1.10)	24	0.92	(0.54-1.56)	104	1.25	(0.94-1.66)
62 Q5	9213	27	1.61	(0.93-2.79)	27	0.81	(0.50-1.32)	35	1.42	(0.88-2.28)	73	0.94	(0.69-1.28)
				p-trend 0.13			p-trend 0.44			p-trend 0.20			p-trend 0.75
continuous, per 25 g/day increment	48223	96	1.20	(0.98-1.46)	137	0.94	(0.77-1.16)	148	1.10	(0.95-1.29)	443	0.97	(0.87-1.08)
Leafy vegetables, cooked													
4 Q1	9505	21	1	reference	30	1	reference	29	1	reference	94	1	reference
12 Q2	10208	22	0.93	(0.51-1.71)	31	0.90	(0.54-1.50)	32	0.95	(0.56-1.59)	101	0.93	(0.69-1.26)
19 Q3	9557	19	0.89	(0.47-1.66)	28	0.93	(0.55-1.57)	32	1.09	(0.65-1.83)	79	0.81	(0.59-1.12) ^d
27 Q4	9803	21	0.96	(0.52-1.78)	24	0.75	(0.43-1.31)	26	0.84	(0.49-1.45)	89	0.90	(0.66-1.23) ^e
42 Q5	9150	13	0.63	(0.31-1.27)	24	0.81	(0.47-1.40)	29	1.01	(0.59-1.71)	80	0.86	(0.62-1.18)
				p-trend 0.23			p-trend 0.38			p-trend 0.93			p-trend 0.37
continuous, per 25 g/day increment	48223	96	0.82	(0.60-1.13)	137	0.90	(0.68-1.19)	148	1.13	(0.85-1.50)	443	0.91	(0.77-1.07) ^d

categorical median (g/day)	Sub-cohort person years at risk	Esophageal squamous cell carcinoma			Esophageal adenocarcinoma			Gastric cardia adenocarcinoma			Gastric noncardia adenocarcinoma			
		cases (n) ^b	RR	(95% CI)	cases (n) ^b	RR	(95% CI)	cases (n) ^b	RR	(95% CI)	cases (n) ^b	RR	(95% CI)	
Leafy vegetables, raw														
Q1	1	9254	24	1	reference	34	1	reference	32	1	reference	101	1	reference
Q2	4	10167	17	0.68	(0.36-1.29)	32	0.87	(0.53-1.43)	27	0.79	(0.46-1.34)	94	0.91	(0.67-1.23)
Q3	7	9573	21	0.88	(0.49-1.60)	29	0.80	(0.48-1.34)	28	0.82	(0.49-1.39)	90	0.89	(0.66-1.21)
Q4	12	9649	22	0.92	(0.51-1.66)	21	0.59	(0.34-1.02)	33	0.99	(0.59-1.63)	76	0.75	(0.55-1.04)
Q5	22	9581	12	0.51	(0.25-1.04)	21	0.60	(0.34-1.05)	28	0.86	(0.50-1.63)	82	0.84	(0.62-1.15)
				p-trend 0.13			p-trend 0.05			p-trend 0.88			p-trend 0.24	
continuous, per 25 g/day increment		48223	96	0.77	(0.36-1.63)	137	0.57	(0.32-1.03)	148	0.90	(0.57-1.43)	443	0.79	(0.59-1.05)
Total fruit														
Q1	43	9508	33	1	reference	39	1	reference	46	1	reference	113	1	reference
Q2	107	10213	22	0.62	(0.36-1.08)	31	0.84	(0.51-1.36)	26	0.61	(0.37-1.01)	90	0.80	(0.59-1.07)
Q3	155	10123	18	0.51	(0.29-0.92)	26	0.72	(0.44-1.21)	28	0.69	(0.42-1.12)	94	0.85	(0.63-1.14)
Q4	215	10368	13	0.35	(0.19-0.67)	22	0.65	(0.38-1.11)	30	0.79	(0.49-1.27)	83	0.74	(0.55-1.00)
Q5	326	10573	15	0.40	(0.21-0.74)	26	0.82	(0.49-1.38)	26	0.76	(0.46-1.25)	80	0.73	(0.54-1.00)
				p-trend 0.002			p-trend 0.36			p-trend 0.50			p-trend 0.06	
continuous, per 25 g/day increment		50785	101	0.92	(0.86-0.97)	144	0.99	(0.95-1.03)	156	0.98	(0.94-1.02)	460	0.98	(0.96-1.01)
Citrus fruits														
C1	0	5048	18	1	reference	30	1	reference	32	1	reference	61	1	reference
C2	8	11169	19	0.48	(0.25-0.93)	35	0.57	(0.34-0.95)	47	0.74	(0.46-1.17)	104	0.81	(0.58-1.14)
C3	33	11494	27	0.67	(0.36-1.24)	24	0.43	(0.24-0.75)	30	0.53	(0.31-0.88)	100	0.81	(0.57-1.14)
C4	77	11490	18	0.43	(0.22-0.85)	29	0.55	(0.32-0.95)	28	0.53	(0.31-0.90)	109	0.89	(0.63-1.25)
C5	156	11583	19	0.46	(0.23-0.89)	26	0.52	(0.30-0.91)	19	0.39	(0.22-0.69)	86	0.72	(0.51-1.03)
				p-trend 0.13			p-trend 0.29			p-trend 0.004			p-trend 0.23	
continuous, per 25 g/day increment		50785	101	0.98	(0.89-1.07)	144	0.96	(0.89-1.04)	156	0.89	(0.81-0.97)	460	0.98	(0.95-1.02)

CI, confidence interval; RR, Incidence Rate Ratio; Q, quintile; T, tertile. ^a The first two years of the follow-up were excluded from all analyses, see text; ^b The number of cases in the vegetable analyses is lower than in the fruit analyses, due to the exclusion of subjects with inconsistent vegetable data; ^c Tests for dose-response trends were assessed by fitting ordinal exposure variables as continuous terms in the Cox proportional hazards model; ^d The proportional hazards assumption was violated for the exposure variable in this analysis. The interaction between the exposure variable and time was statistically significant; ^e The proportional hazards assumption was violated for the exposure variable in this analysis. The interaction between the exposure variable and time was not statistically significant; ^f Due to a large number of nonusers of citrus fruits, categories instead of quintiles were used in analyses: C1 are non-users, C2 to C5 are users, divided into quartiles.

Supplemental Table 8.2

Age- and sex-adjusted associations between consumption of individual vegetable and fruit items and risk of esophageal and gastric cancer subtypes; Netherlands Cohort Study on diet and cancer (NLCS) 1986-2002.^a

Vegetable item	Subcohort person years at risk	Esophageal squamous cell carcinoma			Esophageal adenocarcinoma			Gastric cardia adenocarcinoma			Gastric noncardia adenocarcinoma		
		cases (n) ^b	RR ^c	(95% CI)	cases (n) ^b	RR ^c	(95% CI)	cases (n) ^b	RR ^c	(95% CI)	cases (n) ^b	RR ^c	(95% CI)
String/French beans	48223	96	0.91	(0.62-1.32)	137	1.14	(0.87-1.49)	148	0.84	(0.58-1.21) ^d	443	0.87	(0.71-1.06)
Cauliflower	48223	96	0.87	(0.53-1.44)	137	0.95	(0.56-1.61)	148	0.65	(0.42-1.02)	443	1.01	(0.80-1.27)
Lettuce	48223	96	0.74	(0.31-1.78)	137	0.46	(0.21-1.02)	148	0.95	(0.52-1.71)	443	0.78	(0.54-1.14)
Carrots, cooked	48223	96	0.63	(0.30-1.33)	137	0.85	(0.45-1.60)	148	0.69	(0.36-1.34) ^e	443	0.72	(0.53-0.99)
Endive, cooked	48223	96	0.75	(0.48-1.17)	137	0.92	(0.61-1.38)	148	1.05	(0.72-1.54)	443	0.82	(0.64-1.06)
Brussels sprouts	48223	96	1.24	(0.71-2.17)	137	1.29	(0.63-2.66)	148	0.95	(0.49-1.83)	443	1.17	(0.81-1.68)
Sauerkraut	48223	96	0.61	(0.21-1.79)	137	1.31	(0.58-2.97)	148	0.87	(0.39-1.96)	443	0.99	(0.64-1.52)
Tomatoes	48223	96	0.93	(0.69-1.25)	137	0.76	(0.60-0.96)	148	0.96	(0.75-1.24)	443	1.07	(0.95-1.21)
Onion	48223	96	1.24	(0.95-1.61)	137	0.86	(0.67-1.11)	148	1.19	(0.97-1.47)	443	0.92	(0.80-1.06)
Spinach	48223	96	0.81	(0.45-1.45) ^d	137	0.80	(0.45-1.40)	148	1.37	(0.84-2.24)	443	0.98	(0.73-1.30) ^d
Beetroot	48223	96	0.50	(0.22-1.13)	137	1.03	(0.58-1.81)	148	1.25	(0.79-1.98)	443	0.91	(0.62-1.33)
Kale	48223	96	0.25	(0.04-1.62)	137	0.97	(0.28-3.38) ^d	148	1.12	(0.32-3.92)	443	1.28	(0.60-2.72) ^e
Fruit item													
Apples and pears	50785	101	0.88	(0.80-0.96)	144	1.02	(0.96-1.08)	156	1.02	(0.98-1.07)	460	0.98	(0.94-1.01)
Strawberries	50785	101	0.66	(0.30-1.49)	144	0.77	(0.42-1.42)	156	0.95	(0.54-1.67) ^e	460	0.90	(0.65-1.24)
Oranges and fresh orange juice	50785	101	0.97	(0.87-1.09)	144	0.94	(0.86-1.03)	156	0.86	(0.77-0.95)	460	0.98	(0.94-1.03)

CI, confidence interval; RR, Incidence Rate Ratio. ^a The first two years of the follow-up were excluded from all analyses, see text; ^b The number of cases in the vegetable analyses is lower than in the fruit analyses, due to the exclusion of subjects with inconsistent vegetable data; ^c Continuous variables, RR per 25 g/day increments; ^d The proportional hazards assumption was violated for the exposure variable in this analysis. The interaction between the exposure variable and time was statistically significant; ^e The proportional hazards assumption was violated for the exposure variable in this analysis. The interaction between the exposure variable and time was not statistically significant.

Supplemental Table 8.3

Age- and sex-adjusted associations between consumption of vegetables and fruits and risk of esophageal and gastric cancer subtypes, stratified by sex and cigarette smoking status; Netherlands Cohort Study on diet and cancer (NLCS) 1986-2002^a.

	Subcohort	Esophageal squamous cell carcinoma			Esophageal adenocarcinoma			Gastric cardia adenocarcinoma			Gastric noncardia adenocarcinoma		
		person years at risk	cases (n) ^b	RR (95% CI)	cases (n) ^b	RR (95% CI)	cases (n) ^b	RR (95% CI)	cases (n) ^b	RR (95% CI)	cases (n) ^b	RR (95% CI)	
Total vegetables, RRs per 25 g/day increments													
Sex													
Men	22621	54	0.94 (0.84-1.05)		106	0.99 (0.92-1.07)		125	0.99 (0.93-1.07)		294	0.96 (0.92-1.01)	
Women	25601	42	1.04 (0.96-1.13)		31	0.86 (0.76-0.98)		23	1.04 (0.93-1.17)		149	1.01 (0.95-1.08)	
			p-interaction ^c 0.15			p-interaction 0.06			p-interaction 0.48			p-interaction 0.21	
Cigarette smoking status													
Never smoker	18728	22	1.08 (0.98-1.19)		26	0.98 (0.85-1.13)		24	1.12 (1.00-1.25)		105	1.01 (0.94-1.09)	
Former smoker	17369	28	0.99 (0.86-1.13)		68	1.03 (0.94-1.12)		72	0.96 (0.87-1.05)		180	0.98 (0.93-1.04)	
Current smoker	12126	46	0.95 (0.85-1.06)		43	0.85 (0.74-0.97)		52	1.00 (0.90-1.10)		158	0.96 (0.91-1.01)	
			p-interaction 0.18			p-interaction 0.06			p-interaction 0.12			p-interaction 0.54	
Total fruit, RRs per 25 g/day increments													
Sex													
Men	23722	55	0.87 (0.79-0.96)		112	1.00 (0.95-1.05)		132	0.96 (0.91-1.00)		304	0.98 (0.95-1.01)	
Women	27062	46	0.96 (0.89-1.03)		32	0.98 (0.90-1.06)		24	1.07 (1.01-1.14)		156	1.00 (0.96-1.04)	
			p-interaction 0.11			p-interaction 0.67			p-interaction 0.003			p-interaction 0.45	
Cigarette smoking status													
Never smoker	19774	23	1.01 (0.94-1.08)		28	1.00 (0.92-1.07)		27	1.02 (0.95-1.10)		110	0.99 (0.95-1.03)	
Former smoker	18099	30	0.92 (0.83-1.02)		70	1.02 (0.97-1.08)		74	0.99 (0.94-1.04)		186	1.00 (0.97-1.04)	
Current smoker	12912	48	0.89 (0.80-0.99)		46	0.93 (0.86-1.01)		55	0.95 (0.87-1.04)		164	0.98 (0.93-1.03)	
			p-interaction 0.12			p-interaction 0.16			p-interaction 0.46			p-interaction 0.66	

CI, confidence interval; RR, Incidence Rate Ratio. ^a The first two years of the follow-up were excluded from all analyses, see text; ^b The number of cases in the vegetable analyses is lower than in the fruit analyses, due to the exclusion of subjects with inconsistent vegetable data; ^c p-values for interaction were calculated by including a cross-product term in the Cox proportional hazards model.

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9

General discussion

This chapter contains a discussion of the most important findings described in this thesis in relation to other published research. Methodological points related to the thesis topic are also discussed. In addition, strengths and weaknesses of the study design and analyses are considered. Finally, the implications of our findings, and suggestions for future research will be addressed.

MAIN FINDINGS

In this thesis, we described two studies into lifestyle factors and their association with risk of Barrett's esophagus (BE), using data from the Netherlands Cohort Study on Diet and Cancer (NLCS). We observed that being overweight or obese was a risk factor for BE in women, but not in men. Cigarette smoking moderately increased the risk of BE in both sexes. No association was observed between consumption of alcohol and risk of BE (Chapter 2). In Chapter 3 we described that toenail selenium status was not associated with the development of BE. However, in one subgroup, namely BE patients that progressed to high-grade dysplasia or esophageal adenocarcinoma (EAC), we did observe an inverse association with toenail selenium status. These findings are summarized in Table 9.1.

When we followed patients who were diagnosed with BE, we found that these patients were at increased risk of not only esophageal cancer, but possibly also other cancers, specifically small intestinal and pancreatic cancer. Overall mortality of BE patients was not increased (Chapter 4).

In Chapter 5, we used data from European cancer registries to study trends in incidence of esophageal and gastric cancers during the 1983-1997 period. Adenocarcinomas of the esophagus and gastric cardia rose in incidence in most countries, the strongest in the UK and Ireland. Incidence of esophageal squamous cell carcinoma (ESCC) rose in women from all countries, and in men from Northern Europe and Slovakia, but declined mostly in men from Southern and Western Europe. In nearly all countries, the incidence of gastric non-cardia adenocarcinomas (GNCA) declined.

In the NLCS, we found that alcohol consumption was related to a strong increase in risk of ESCC, while it was not related to risk of EAC, gastric cardia adenocarcinoma (GCA), and GNCA (Table 9.1). Cigarette smoking clearly increased the risk of all four cancers. Furthermore, combined exposure to alcohol and cigarette smoking was associated with a very strong increase in ESCC risk, compatible with a multiplicative interaction model (Chapter 6). By contrast, a strong inverse association was described for higher toenail selenium status and risk of ESCC. A less strong inverse association was observed with GCA risk, while toenail selenium status was associated with EAC risk only in some groups: women, never smokers, and people with low antioxidant consumption (Chapter 7). Finally, Chapter 8 described the association between consumption of vegetables and fruits and risk of esophageal and gastric cancer subtypes. Associations were generally inverse, and some statistically significant inverse

associations were found with risk of ESCC, EAC, and GCA for specific vegetables and fruits (Table 9.1). Specifically in smokers, vegetables and fruits consumption was inversely associated with ESCC and EAC risk.

Table 9.1 Main findings of the etiological studies described in this thesis.

Risk factor	Direction and strength of observed association					Chapter
	BE	ESCC	EAC	GCA	GNCA	
Overweight	Men: 0 Women: +	–	++	++	–	2, Merry et al. ¹
Cigarette smoking	+	++	++	+	+	2, 6
Alcohol consumption	0	++	0	0	0	2, 6
Toenail selenium status	0	--	–	–	Not studied	3, 7
Vegetables consumption	Not studied	–	–	–	0	8
Fruits consumption	Not studied	–	–	–	0	8

++ strong positive association (RRs ≥ 2.0), + positive association, 0 no association (RRs very close to 1 and no dose-response trends), – inverse association, -- strong inverse association (RRs ≤ 0.5).

STRENGTHS OF THE NETHERLANDS COHORT STUDY

The Netherlands Cohort Study has a prospective cohort study design, which brings several advantages. Prospective cohort studies are less susceptible to information and selection bias compared with case-control studies. The validity of the former study design is therefore usually higher. A second major strength of the prospective character is the possibility to exclude cancer cases who were diagnosed early during follow-up. This exclusion can diminish or eliminate bias caused by reverse causation. Reverse causation can specifically be a problem in case-control studies on esophageal and gastric cancer, as we argued before.^{2,3} Third, in the NLCS there is much information available on potentially confounding variables. If necessary, we used this information to adjust the associations between risk factors and disease. This adjustment further increased the validity of our results.

DIFFERENCES BETWEEN ESOPHAGEAL AND GASTRIC CANCER TYPES

Based on the observed differences in time trends and risk factors, we understand more and more that ESCC and EAC are two different diseases and should be investigated as such. The same is true for gastric cancer, which is also more and more often being viewed as two separate diseases: GCA and GNCA. Below we will discuss the differences

we observed in the light of findings by others. Also, some methodological issues related to our research into ESCC, EAC, GCA, and GNCA will be dealt with.

ESCC versus EAC

In our trend study, we observed differences in incidence trends for ESCC and EAC in many European countries. Incidence of EAC rose in most countries, while the incidence trends were mixed for ESCC. As mentioned in Chapter 1, this may indicate a different etiology of these two histologic types of esophageal cancer. ESCC and EAC also differ with respect to pathology, tumor biology, and prognosis. This is acknowledged in the newest TNM staging system, that provides separate stage groupings for ESCC and adenocarcinomas of the esophagus and esophagogastric junction.⁴ ESCC patients are on average somewhat younger than EAC patients.^{5,6} Also, some studies have reported a better prognosis of EAC than of ESCC, particularly in early stage disease.^{5,6}

In our studies on risk factors for ESCC and EAC, summarized in Table 9.1, we observed differences for overweight, alcohol consumption, and toenail selenium status. These differences are in agreement with observations in other studies. Positive associations are found between overweight and EAC, and inverse associations with ESCC.⁷⁻⁹ Alcohol consumption is only associated with increased risk of ESCC, not with EAC.^{8,10,11} Because our study is the first to investigate selenium and EAC risk, we cannot compare our findings with the literature yet. We consequently recommend others to study selenium and EAC risk.

ESCC and EAC were similar with respect to their associations with cigarette smoking, vegetables consumption, and fruits consumption. Other cohort studies also found increased risks of ESCC and EAC for smokers,^{8,10,12} although in two out of three studies the risks were higher for ESCC than for EAC.^{10,12} In the literature, inverse associations with vegetables and fruits consumption are reported somewhat more frequently for ESCC than for EAC.^{7,13-16}

From the above, we can conclude that ESCC and EAC are different with regard to various aspects: risk factors, incidence trends, pathology, tumor biology, and prognosis. We therefore believe that it is not informative to study esophageal cancers as one entity. ESCC and EAC should be regarded as two distinct diseases in future research.

EAC versus GCA

When making a comparison between EAC and GCA, one has to consider the classification of these cancers. EAC most commonly arises in the distal part of the esophagus, which is close to the gastric cardia. If an esophageal tumor is located near the gastroesophageal junction, it can grow into the cardia. The opposite is also possible: a tumor that originates from the gastric cardia can grow into the distal esophagus. At the time of diagnosis, however, it is sometimes impossible to determine whether a cancer has arisen from the distal esophagus or from the proximal

stomach.^{17,18} Sometimes, a tumor is then classified as gastroesophageal junction cancer.¹⁷

The cancer incidence data used in our study come from the Netherlands Cancer Registry. The Netherlands Cancer Registry is a high quality cancer registry. Specially trained registration clerks register directly from the medical records in the hospitals. Clinical information as well as pathology information are collected.¹⁹⁻²² The classification of EAC and GCA is based on these cancer registry data.

When our findings on EAC and GCA are compared, we can see that these cancers share all risk factors that we investigated, i.e., overweight, smoking, alcohol consumption, toenail selenium status, and vegetables and fruits consumption (Table 9.1). These findings are in agreement with other studies, which also found similarities in risk factors for EAC and GCA.^{e.g. 8-10,14, 23}

Data on trends in incidence are not separately presented for EAC and GCA in the majority of trend studies. This has to do with the fact that in the past, adenocarcinomas of the lower esophagus had to be coded as cardia tumors, according to version 9 of the International Classification of Diseases. Also, researchers may not want to separate these cancers, because of possible misclassification.

To sum up, it may be said that in our studies based on high quality cancer registry data, we observed that EAC and GCA are etiologically very similar diseases. Further, clinically it is very difficult to distinguish these diseases. In view of these facts, we believe it may not be useful to keep trying to separate EAC and GCA, and therefore recommend treating these diseases as one entity: gastroesophageal junction adenocarcinomas.

GCA versus GNCA

In this thesis, we also found some differences between GCA and GNCA. Trends in incidence rates were strikingly different for adenocarcinomas of the esophagus and gastric cardia than for GNCA (the latter separately analyzed as gastric cancers of other specified sites and not otherwise specified gastric cancers) (Chapter 5). This observation is in agreement with studies on trends in gastric cancer subtypes in other countries.^{e.g. 24-28}

The associations we observed in the etiological analyses were indeed different for GCA and GNCA for some risk factors (Table 9.1), although the contrast is less sharp than for ESCC versus EAC. The largest difference was present for overweight, which was a strong risk factor for GCA but inversely associated with GNCA. Recent data from the prospective AARP Diet and Health study confirm our findings: increased BMI was found to be a risk factor for GCA, but not for GNCA.²⁹ We are unsure whether toenail selenium status is differently associated with risk of GCA than with risk of GNCA. In a previous study, we found that selenium was inversely associated (not statistically significant) with risk of total gastric cancer, but we did not report on GNCA separately.³⁰ Possibly, selenium is only inversely associated with GCA, and not with

GNCA. This was observed by a prospective study in Linxian, China.²³ Further, the associations between consumption of vegetables and fruits were inverse for GCA, while this was not the case for GNCA. Other prospective studies have generally found few associations between vegetables and fruits consumption and risk of GCA as well as GNCA.^{7,14,31-33}

As mentioned in several chapters, we lack information on infection with *Helicobacter* (H.) *pylori*. On this subject, case-control studies have an advantage over cohort studies. Medical data, including data on H. *pylori* infection, are more readily available in case-control studies. One reason is that these studies are often carried out in a medical setting, in contrast to the general population setting of cohort studies. Also, the number of participants is usually much lower in case-control studies than in cohort studies.

The lack of data on H. *pylori* is particularly important for GNCA, as H. *pylori* is the main risk factor for this cancer. A recent review of epidemiologic studies that investigated interaction between H. *pylori* infection and other risk factors for gastric cancer concluded that there is a suggestion of a positive interaction between H. *pylori* infection and smoking on gastric cancer risk. Further, the potential protective effect of dietary antioxidants seemed to be observed only in subjects infected with H. *pylori*, although the results were inconsistent.³⁴ Analyzing the interaction between H. *pylori* and other risk factors for GNCA within prospective studies would be of interest.

In accordance with our belief that it is not informative to study esophageal cancer as one entity, we neither believe it is informative to study gastric cancer as one entity. This thesis shows that GCA and GNCA are quite different diseases with respect to their etiology, and future research should therefore make a distinction between these two diseases.

BARRETT'S ESOPHAGUS VERSUS ESOPHAGEAL ADENOCARCINOMA

Besides making comparisons between subtypes of esophageal and gastric cancer, an important focus of this thesis is also to make a comparison between BE and EAC. We believe this thesis contributes significantly to the knowledge of these two diseases, also because the evidence is based on prospective data. The existence of the nationwide network and registry of histopathology and cytopathology in the Netherlands (PALGA)³⁵ offered us the unique possibility to research BE in a prospective cohort study.

The results of the etiologic analyses on BE and EAC clearly show some differences between these diseases. In our cohort, overweight, cigarette smoking, and toenail selenium status all have a different association with BE than with EAC (Table 9.1). It seems that the associations found for EAC are less strong or not present for BE, at least for the risk factors described in this thesis. In the literature, obese persons are

generally found to have 3 times more risk of BE as well as EAC, and overweight persons are found to have 1.5 times the risk of BE and EAC compared with normal weight persons.³⁶⁻⁴¹ These risk estimates for BE come from case-control studies. The only other cohort study found no association between BMI and BE risk.⁴² Therefore, the inconsistency between our results and most other studies may be due to the study design. Smoking is probably more strongly associated with EAC compared with BE, although the exact risk estimates vary between studies.^{8,10,37,43-45} For selenium and risk of BE and EAC, there are no other studies available for comparison. It would thus be interesting if others examined these associations.

The association between vegetables and fruits consumption and risk of BE is not described in this thesis, but is certainly worthwhile investigating given the inverse associations we observed with EAC risk. More in general, epidemiological studies on (lifestyle) risk factors for BE are requested as the evidence is quite limited to date. It also remains important to compare BE and EAC, to be able to find clues about the etiologically relevant time window for exposures.

In our analyses of risk factors for BE and EAC, we were able to check for possible confounding effects of various dietary and lifestyle habits of our cohort participants. No information, however, was available on the presence of gastroesophageal reflux disease (GERD). Use of medications for the treatment of reflux, such as antacids, histamine 2 receptor antagonists (H2RA), and proton pump inhibitors (PPI), were not measured well in our cohort. Here, case-control studies may have an advantage over cohort studies, because this medical information is generally more readily available in these studies. GERD or reflux symptoms may be confounders, effect-modifiers, or intermediates in the associations we investigated. Therefore, future studies are recommended to evaluate the role of GERD and reflux. This can best be done by comparing the results from different statistical models, with and without adjustment for these factors. This approach has already been applied by some researchers.^{43,46}

Another point of interest is that there are some foods that have been associated with temporary symptoms of reflux. These include dietary fat, chocolate, mints, coffee, onions, citrus fruits, and tomatoes.⁴⁷ Persons already suffering from reflux symptoms may therefore try to avoid eating these foods in order to reduce their complaints. This may influence not only our study, but all studies investigating diet and risk of BE, because the dietary habits of BE cases (who often have a history of reflux) will then be different from the habits of the non-cases. Specifically, cases may consume less reflux-inducing foods than non-cases. In this way, reverse causation may play a role in the observed associations between diet and risk of BE. We attempted to remove the effects of reverse causation from our results by excluding BE cases that were diagnosed in the first 2 years of the follow-up. These patients may be the patients that already had considerable complaints at the time they filled out our baseline food frequency questionnaire. These complaints may have influenced their diet and might also have led to their diagnosis of BE. Nevertheless, excluding these early cases may

not have solved the problem entirely, as reflux symptoms can already be present a long time before BE diagnosis.^{48,49}

The paradox of Barrett's esophagus

It has recently been proposed that BE is a successful adaptation to the harsh intra-esophageal environment of chronic gastroesophageal reflux disease. The BE tissue has several functions not present in the normal squamous cell epithelium, that protect against further reflux injury.⁵⁰ Besides being a successful adaptation, BE is also associated with increased risk of EAC, which is the reason that clinicians and researchers are interested in BE. This increased risk of EAC is also the reason for regular endoscopic surveillance of BE patients, to try to detect progression to EAC in an early stage.^{51, 52} As mentioned before, the incidence of this highly lethal cancer is rising in the Western world. Making a valid estimate of the risk of EAC in BE patients is therefore essential for these patients, the cost-efficiency of surveillance, and the relevance of BE as a precancerous lesion. Quite some studies have tried to estimate the risk, but there are quite large differences between the estimates from different studies.⁵³ A recent meta-analysis estimated the risk of esophageal adenocarcinoma in BE patients to be between 4.1 and 6.1% per 10 year.⁵³ In our study described in Chapter 4, we have therefore tried to make a valid estimate of, among other things, EAC risk in BE using a population-based design. Our estimate of the incidence rate was 3.7% per 10 year, which is very close to the estimate from the meta-analysis.

Even though BE patients have a risk of EAC that is equal to or larger than 10 times the risk of the general population (Chapter 4), their absolute risk of EAC is still quite low. The majority of BE patients never develop EAC.⁵³ Thus, one could say there is some overdiagnosis of BE. This overdiagnosis contrasts sharply to the underdiagnosis of early stage EAC. A large proportion of persons diagnosed with EAC has not had any previous complaints like reflux (50%), has not been diagnosed with GERD (80%), and did not have a prior diagnosis of BE (95%).⁵⁴ Because of the absence of early complaints and diagnosis of precursor lesions, these persons are not under surveillance and therefore the EAC is detected very late. By that time, the cancer is already advanced and the associated mortality high.

Reid et al. have recently nicely described this contrast between overdiagnosis of BE and underdiagnosis of early EAC, which they named the paradox of Barrett's esophagus.⁵⁴ Changing this paradox of BE is necessary to improve patient care and lower esophageal cancer mortality. One possibility to achieve this, is to identify those patients with BE that are at highest risk of progression, thus making a risk stratification. In our follow-up study in Chapter 4, we observed that male BE patients have a higher risk of progression to EAC than women. This was also reported in a meta-analysis on risk of progression in BE.⁵³ Also, BE patients with high-grade dysplasia (HGD) were at greatest risk of progression. However, the assessment of the grade of dysplasia (negative, indefinite, low grade, or high grade)⁵⁵ is subject to a large inter-

observer variation.⁵⁴ This assessment may thus be improved. Several researchers have studied biomarkers that may be valuable for risk stratification in BE. These biomarkers include measures of genomic instability, chromosomal alterations, chromosomal instability, microsatellite instability, mutations, and disruption of regulatory pathways in BE tissue.⁵⁴ Future use of these biomarkers in clinical practice may be promising. Besides these biomarkers, other characteristics of the BE patient, such as demographic, lifestyle and medical characteristics might also be useful for risk stratification. However, the associations between these factors and risk of progression of BE remain to be well assessed. Valuable information on this topic could be obtained by setting up a prospective cohort of BE patients. At the start of that cohort study, information on all kinds of possibly relevant factors for progression should be collected.

Ultimately, the results of all these investigations could be used to develop a risk score that clinicians can use to predict the individual risk of progression in their BE patient, communicate this risk to the patient, and apply personalized patient care.⁵⁶ Patient care may include endoscopic surveillance, endoscopic treatment, chemoprevention with NSAIDs/aspirin, and advice to make lifestyle changes. For patients at low risk of progression, no intervention at all may be the best care. However, these options remain to be demonstrated as effective in trials.⁵⁴

IMPLICATIONS FOR PUBLIC HEALTH

Naturally, the lifestyle factors described in this thesis are not only related to risk of Barrett's esophagus and esophageal and gastric cancer subtypes. Risk of many other diseases is influenced (increased or decreased) by smoking, overweight and obesity, alcohol consumption and dietary habits. The directions of the associations we observed with BE and esophageal and gastric cancers generally correspond to the direction of the associations with other diseases (Table 2). As a consequence of these diseases, individuals live some years of their lives in poor health. Further, a number of life years are lost due to early mortality. The National Institute for Public Health and the Environment (RIVM) in the Netherlands has quantified the contribution of several risk factors to the burden of disease (Table 2).^{57, 58} For example, compared with never smokers, smokers lose on average 4.1 life years and 4.6 healthy life years. Overweight and obesity also cause significant loss of life years and healthy life years, while the effects of excess alcohol consumption are more limited. No figures were available for the effect of selenium status and vegetables and fruit consumption.

These figures stress the importance of recommendations and guidelines for healthy living, as described by e.g. the World Cancer Research Fund⁵⁹, the National Health Council of the Netherlands,⁶⁰ and the Dutch Cancer Society.⁶¹ Of course, only formulating these recommendations and guidelines is not sufficient. It is not always self-evident or easy for individuals to follow these recommendations and therefore

they should be supported.⁵⁸ The findings of the research described in this thesis underline the guidelines for a healthy lifestyle and the importance of motivating and helping the population to comply with these guidelines.

Table 9.2 Effects of risk factors on life years and healthy life years (with 95% confidence intervals) (Chronic Diseases Model).^a

Risk factor	Life years lost	Healthy life years lost (HALE) ^b
Individuals in the risk groups:		
Smokers (including former smokers)	4.1 (3.7-4.6)	4.6 (4.1-5.4)
Overweight subjects ^c	1.2 (1.0-1.5)	2.1 (1.8-2.3)
Obese subjects ^c	3.0 (2.3-3.6)	5.1 (4.5-5.6)
Users of excess alcohol ^d	0.6 (0.5-0.7)	0.9 (0.8-1.0)

^a Adapted from reference ⁵⁷; ^b HALE, health-adjusted life expectancy: a measure for the number of healthy life years someone can expect to live from a certain age. This is calculated based on the prevalence and the seriousness of diseases; ^c Overweight is defined as BMI ≥ 25 kg/m². Obesity is defined as BMI ≥ 30 kg/m². The reference group is normal weight (BMI 18.5-25 kg/m²); ^d Defined as ≥ 3 glasses of alcohol per day for men, and ≥ 2 glasses of alcohol per day for women.

SUGGESTIONS FOR FUTURE RESEARCH

Besides the suggestions for future research described in the texts above, below we formulated a few additional recommendations.

It is important to keep monitoring the incidence of histologic types of esophageal cancer and subsites of gastric cancer. Also in countries in which no changes have been observed yet, these can still take place later. Preferably, figures of recent date should be used in trends studies. Observed trends can further be extrapolated to make predictions about future trends. Results from trends studies can give clues about yet unknown risk factors, and moreover they can be used for e.g. future health care planning.

An exposure of interest that could be investigated further is physical activity, which might reduce risk of esophageal and gastric cancers independently of BMI.^{62, 63} Additionally, reproductive and hormonal factors may be related to risk of esophageal and gastric cancer subtypes and may also explain the high male predominance of EAC and GCA. Only a few studies have been conducted on this topic and this requires further attention.^{64, 65}

Finally, besides studying BE, studying precursor lesions of other esophageal and gastric cancer subtypes (ESCC, GCA, and GNCA) may be valuable. Issues that deserve particular attention are the diagnosis and definition of the precursor lesion, risk factors for the development and malignant progression of the lesion, and the magnitude of the risk of progression to cancer.

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SUMMARY

Cancer affects millions of persons in the world. In the Netherlands, cancer has become the most important cause of death in 2008. This thesis concerns two types of cancer: cancer of the esophagus, and cancer of the stomach. Esophageal and gastric cancer are diseases with a poor prognosis: the 5-year survival rate is only 14% for esophageal cancer and 21% for gastric cancer. Besides these cancers, this thesis also concerns Barrett's esophagus (BE). BE is a condition of the lower esophagus, in which the normal squamous epithelium is replaced by columnar epithelium. Patients with BE are at increased risk of developing esophageal adenocarcinoma (**Chapter 1**).

There are some indications that the two main subtypes of esophageal cancer, esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC), have a different etiology. This is also true for the two subtypes of gastric cancer: gastric cardia adenocarcinoma (GCA) and gastric non-cardia adenocarcinoma (GNCA). First, esophageal and gastric cancer subtypes are geographically differently distributed. Second, the trends in incidence rates of these cancer subtypes differ. Third, the male-to-female ratio of the incidence of these cancers differs by subtype.

In this thesis, we investigated whether the risk factors for ESCC, EAC, GCA, and GNCA are indeed different. We studied cigarette smoking, alcohol consumption, overweight, toenail selenium status, and consumption of vegetables and fruits. Further, we also looked into the association of these factors with the risk of BE. These lifestyle factors can potentially be modified and can thus have a role in primary prevention.

The research described in this thesis is based on a prospective cohort study: the Netherlands Cohort Study on diet and cancer (NLCS). The NLCS was initiated in September 1986, with the enrollment of 120,852 men and women aged 55-69 years. These subjects were randomly selected from Dutch municipal population registries. At baseline, all subjects filled out a questionnaire on dietary habits, lifestyle, and other risk factors for cancer. According to the case-cohort approach, data are processed and analyzed for a random sample of the cohort (the subcohort) and cases. The subcohort consists of 5,000 men and women who were sampled at baseline. This subcohort is followed-up for vital status and migration and is used to estimate the person-time at risk for the total cohort. Incident cases of BE and cancer were identified in the whole cohort. BE cases were identified through record linkage with data from PALGA (the nationwide registry for histopathology and cytopathology in the Netherlands). The existence of this unique nationwide pathology registry offered us the opportunity to study BE within a prospective cohort study. A pathologist reviewed and coded further characteristics of the BE cases from the PALGA data. Incident cancer cases were identified through record linkages with PALGA and the Netherlands Cancer Registry. The cohort has been followed-up for vital status through record linkage with the Central Bureau of Genealogy and automated municipal population registries. For deceased cohort members we also obtained information on the cause of death from

Statistics Netherlands. The research described in this thesis was based on 16.3-year follow-up data of the cohort (September 17, 1986 through December 31, 2002).

Chapter 2 describes the association between overweight, smoking, and alcohol consumption and the risk of BE. Overweight and obesity were associated with an increased risk of BE in women, but not in men. The association in women was not specifically due to abdominal overweight. Former cigarette smokers were at 30% increased risk of BE, but current smokers were not. Smoking duration showed a positive association with BE risk, while alcohol consumption was not associated with an increased risk of BE.

A study on toenail selenium status and its relationship with risk of BE is presented in **Chapter 3**. We did not find an association between selenium and risk of BE. No association was found either in subgroups defined by sex, smoking status, body mass index, or intake of antioxidants. For BE cases that later progressed to high-grade dysplasia or adenocarcinoma, we observed an inverse association with selenium.

In **Chapter 4** we used the data of the NLCS to follow BE patients for incidence of esophageal and gastric cancer, other cancers, and cause-specific mortality. We found that esophageal and gastric cancer occurred 10 and nearly 2 times more frequently, respectively, in BE patients than in the general population. Total incidence of all other cancers was 30% increased in BE patients. Small intestinal and pancreatic cancer incidence were increased in particular. All-cause mortality and cause-specific mortality were not increased.

For the study in **Chapter 5**, we used data from the European Network of Cancer Registries. We described the incidence trends of esophageal and gastric cancers in 13 European countries in the period 1983-1997. The incidence of adenocarcinomas of the esophagus and gastric cardia rose in most, but not all, countries, mostly 1-7% per year. Incidence of ESCC rose in women from all countries and in men from Northern Europe and Slovakia, but declined mostly in men from Southern and Western Europe. In nearly all countries, the incidence of GNCA declined.

The results described in **Chapter 6** indicate that consumption of 3 or more glasses alcoholic beverages per day was related to a 4- to 5-fold increase in risk of ESCC. Alcohol consumption was not related to risk of EAC, GCA, and GNCA, while cigarette smoking increased the risk of all four cancers 1.5 to 2 times. Furthermore, combined exposure to alcohol and cigarette smoking was associated with a very strong increase in ESCC risk: up to 8 times compared with people who did not drink or smoke.

In **Chapter 7**, the results are presented for the association between toenail selenium status and risk of ESCC, EAC, and GCA. These results indicate that a high selenium status is associated with a strong decrease in risk of ESCC. An inverse association was also found for GCA. For EAC, inverse associations with selenium were found in subgroups: women, never smokers, and persons with a low antioxidant intake.

The relation between consumption of vegetables and fruits and risk of ESCC, EAC, GCA, and GNCA is described in **Chapter 8**. The results generally indicated inverse

associations. Specifically, raw vegetables were associated with a lower EAC risk and Brassica vegetables were associated inversely with GCA risk. Citrus fruits were inversely associated with EAC and GCA risk. Specifically for current smokers, vegetables and fruits intake was inversely associated with ESCC and EAC risk. Consumption of vegetables and fruits may therefore protect against development of esophageal and gastric cancer subtypes.

This thesis concludes with a discussion of the findings described in this thesis (**Chapter 9**). We make comparisons between ESCC and EAC, and conclude that these cancers are different with regard to various aspects, among others risk factors and incidence trends. GCA and GNCA are also compared, and here we also draw the conclusion that these cancers are different in these respects. Because of these observed differences, we believe it is neither informative to study esophageal cancer as one entity nor to study gastric cancer as one entity in etiologic research. Therefore, we recommend separate analyses of ESCC, EAC, GCA, and GNCA in future studies. Further, it appears that EAC and GCA are quite similar diseases that are very difficult to distinguish. It may therefore not be useful to keep trying to separate these, and we therefore suggest treating these diseases as one entity: gastroesophageal junction adenocarcinomas. Finally, we compare BE and EAC. It appears that the associations found for EAC are less strong or not present for BE, for the risk factors described in this thesis. A comparison between BE and EAC in future studies is valuable, as it may give clues about the etiologically relevant time window for exposures. More - preferably high quality - research into the relation between lifestyle factors and risk of development and progression of BE is needed, as data are still very limited.

SAMENVATTING

Kanker treft miljoenen mensen in de wereld. In Nederland is kanker in 2008 de belangrijkste doodsoorzaak geworden. Dit proefschrift gaat over twee vormen van kanker: slokdarmkanker en maagkanker. Slokdarm- en maagkanker zijn ziekten met een slechte prognose: de 5-jaarsoverleving is slechts 14% voor slokdarm- en 21% voor maagkanker. Naast deze kankers gaat dit proefschrift ook over Barrett's slokdarm. Barrett's slokdarm is een aandoening van het onderste deel van de slokdarm, waarbij het normale plaveiselepitheel is vervangen door cilinderepitheel. Patiënten met Barrett's slokdarm hebben een verhoogd risico op het ontwikkelen van adenocarcinoom van de slokdarm (**Hoofdstuk 1**).

Er zijn aanwijzingen dat de twee belangrijkste vormen van slokdarmkanker, plaveiselcelcarcinoom (PC) en adenocarcinoom (AC), een verschillende ontstaansgeschiedenis hebben. Dit geldt ook voor de twee vormen van maagkanker: kanker van de cardia van de maag (CC) en kanker van andere delen van de maag (NCC). Ten eerste zijn de vier vormen van slokdarm- en maagkanker anders verdeeld over de wereld. Ten tweede zijn er verschillen in de tijdstrends in het vóórkomen van deze vormen van kanker. Ten derde is de man-vrouw verhouding van het vóórkomen van deze vormen van kanker verschillend.

In dit proefschrift onderzochten we of de risicofactoren voor PC, AC, CC en NCC inderdaad verschillend zijn. We bestudeerden het roken van sigaretten, alcohol consumptie, overgewicht, seleniumgehalte in teennagels, en groente- en fruitconsumptie. Verder bekeken we ook het verband tussen deze risicofactoren en de kans op het krijgen van Barrett's slokdarm. Deze leefstijlfactoren kunnen mogelijk worden veranderd en kunnen daarom een rol spelen bij het voorkómen van ziekten.

Het onderzoek dat is beschreven in dit proefschrift is voornamelijk gebaseerd op een prospectief cohortonderzoek: de Nederlandse Cohort Studie naar voeding en kanker (NLCS). De NLCS is in 1986 gestart met 120.852 mannen en vrouwen van 55-69 jaar oud. Deze deelnemers zijn willekeurig geselecteerd uit Nederlandse bevolkingsregisters. Aan het begin van de studie hebben alle deelnemers een vragenlijst ingevuld die ging over voedingsgewoonten, leefstijl en andere risicofactoren voor kanker. De verwerking en analyse van de onderzoeksgegevens gebeurde volgens de 'case-cohort' benadering. Daarbij worden de kankergevallen uit het gehele cohort geanalyseerd samen met het subcohort. Dit subcohort bestaat uit een willekeurige steekproef van 5.000 mannen en vrouwen, die aan het begin van de studie getrokken is uit het cohort. Het subcohort wordt opgevolgd voor informatie over overlijden en emigratie en het wordt gebruikt om de persoonstijd 'at risk' in het gehele cohort te schatten. Nieuwe gevallen van Barrett's slokdarm en kanker worden opgespoord in het gehele cohort. De identificatie van patiënten met Barrett's slokdarm vond plaats door koppeling met gegevens van PALGA (Pathologisch-Anatomisch Landelijk Geautomatiseerd Archief). Het bestaan van dit unieke landelijke archief gaf ons de mogelijkheid om Barrett's slokdarm te bestuderen in een prospectief

cohortonderzoek. Een patholoog bekeek de PALGA gegevens en codeerde verdere kenmerken van de patiënten met Barrett's slokdarm. Het cohort is ook opgevolgd voor informatie over nieuwe kankergevallen, die verkregen is via PALGA en de Nederlandse Kanker Registratie. Via het Centraal Bureau voor de Genealogie en het bevolkingsregister (GBA) is informatie verkregen over welke deelnemers zijn overleden. Met betrekking tot overleden cohortleden hebben we van het Centraal Bureau voor de Statistiek informatie verkregen over de doodsoorzaak. Voor het in dit proefschrift beschreven onderzoek is het cohort gedurende 16,3 jaar gevolgd (17 september 1986 t/m 31 december 2002).

Hoofdstuk 2 beschrijft het verband tussen overgewicht, roken en alcohol consumptie en het risico op Barrett's slokdarm. Overgewicht en obesitas waren geassocieerd met een verhoogd risico op Barrett's slokdarm bij vrouwen, maar niet bij mannen. Het verband bij vrouwen werd niet specifiek veroorzaakt door overgewicht in de buik. Ex-rokers hadden 30% meer risico op Barrett's slokdarm, maar er was geen hoger risico voor huidig rokers. Rookduur verhoogde het risico op Barrett's slokdarm, terwijl alcohol consumptie niet geassocieerd was met een verhoogd risico op Barrett's slokdarm.

Onze studie naar het seleniumgehalte van teennagels en de relatie met risico op Barrett's slokdarm wordt gepresenteerd in **Hoofdstuk 3**. We vonden geen verband tussen selenium en risico op Barrett's slokdarm. Ook vonden we geen verband in subgroepen gedefinieerd op basis van geslacht, rookstatus, overgewicht, of inname van antioxidanten. Voor patiënten met Barrett's slokdarm waarbij later voortgang werd gezien naar hooggradige dysplasie of AC vonden we een beschermend verband met selenium.

In **Hoofdstuk 4** gebruikten we de NLCS gegevens om patiënten met Barrett's slokdarm te volgen voor informatie over het optreden van slokdarm- en maagkanker, andere kankers, en oorzakspecifieke sterfte. We vonden dat slokdarm- en maagkanker respectievelijk 10 en bijna 2 keer zoveel voorkwamen bij patiënten met Barrett's slokdarm als bij de algemene bevolking. Het vóórkomen van totaal kanker (exclusief slokdarm- en maagkanker) was 30% verhoogd onder patiënten met Barrett's slokdarm. Dunne darm- en alvleesklierkanker kwamen vaker voor. Totale sterfte en oorzakspecifieke sterfte waren niet verhoogd.

Voor de studie beschreven in **Hoofdstuk 5** gebruikten we gegevens van het Europese Netwerk van Kanker Registraties. We beschreven de trends in het vóórkomen van slokdarm- en maagkanker in 13 Europese landen in de periode 1983-1997. Het vóórkomen van adenocarcinomen van de slokdarm en cardia van de maag steeg in de meeste, maar niet alle landen, meestal 1-7% per jaar. Het vóórkomen van PC steeg bij vrouwen uit alle landen en bij mannen uit Noord-Europa en Slowakije, maar daalde meestal bij mannen uit Zuid- en West-Europa. In bijna alle landen nam het vóórkomen van NCC af.

De resultaten die beschreven zijn in **Hoofdstuk 6** geven aan dat het drinken van 3 of meer glazen alcoholische drank per dag was gerelateerd aan een 4- tot 5-maal

verhoogd risico op PC. Alcoholconsumptie was niet gerelateerd aan het risico op AC, CC en NCC. Het roken van sigaretten verhoogde het risico op alle vier vormen van kanker 1.5 tot 2 maal. Bovendien was een gecombineerde blootstelling aan alcohol en sigaretten geassocieerd met een zeer sterke verhoging van het risico op PC: tot 8 maal vergeleken met mensen die niet dronken noch rookten.

In **Hoofdstuk 7** worden de resultaten gepresenteerd voor het verband tussen het seleniumgehalte van teennagels en risico op PC, AC en CC. Deze resultaten geven aan dat een hoog seleniumgehalte het risico op PC sterk verlaagt. Een beschermend effect werd ook gevonden voor CC. Voor AC werden er specifiek bij vrouwen, nooit rokers en mensen met een lage inname van antioxidanten beschermende effecten van selenium gevonden.

De relatie tussen groente- en fruitconsumptie en risico op PC, AC, CC en NCC is beschreven in **Hoofdstuk 8**. De resultaten geven over het algemeen beschermende effecten aan. Specifiek werden verbanden gevonden tussen rauwe groenten en een lager AC risico en koolsoorten en een lager CC risico. Citrusfruit verlaagde het risico op AC en CC. Specifiek bij huidige rokers waren groente- en fruitconsumptie geassocieerd met een lager risico op PC en AC. Consumptie van groente en fruit zou kunnen beschermen tegen het krijgen van vormen van slokdarm- en maagkanker.

Dit proefschrift eindigt met een discussie van de beschreven bevindingen (**Hoofdstuk 9**). We maken een vergelijking tussen PC en AC en concluderen dat deze kankers verschillend zijn op diverse aspecten, onder andere risicofactoren en tijdstrends in het voorkomen. CC en NCC worden ook vergeleken en ook hier trekken we de conclusie dat deze kankers verschillend zijn op deze aspecten. Vanwege de verschillen die wij hebben gevonden, geloven wij dat het noch informatief is om slokdarmkanker als één ziekte te bestuderen, noch om maagkanker als één ziekte te bestuderen, wanneer men de risicofactoren onderzoekt. Daarom stellen we voor om in toekomstige studies aparte analyses te doen voor PC, AC, CC en NCC. Verder lijkt het dat AC en CC zeer vergelijkbare ziekten zijn, die moeilijk te onderscheiden zijn. Daarom is het misschien niet zinvol om te blijven proberen deze te scheiden. Vandaar bevelen we aan om deze ziekten als één te beschouwen: adenocarcinomen van de slokdarm-maag overgang. Als laatste vergelijken we Barrett's slokdarm met AC. Het blijkt dat de verbanden die we vonden voor AC, minder sterk of afwezig waren voor Barrett's slokdarm, voor de in dit proefschrift beschreven risicofactoren. Het vergelijken van Barrett's slokdarm en AC in toekomstige studies is waardevol, omdat het aanwijzingen kan geven over de periode die relevant is voor blootstellingen. Meer - bij voorkeur kwalitatief hoogstaand - onderzoek naar de relatie tussen leefstijlfactoren en het risico op het ontwikkelen en de voortgang van Barrett's slokdarm is nodig, want de gegevens zijn nu erg beperkt.

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Jessie

Curriculum Vitae / List of publications

CURRICULUM VITAE

Jessie Steevens was born on December 14, 1981 in Breda, the Netherlands. She completed secondary school (Gymnasium) at the Mencia de Mendoza lyceum in Breda in 2000. That year, she started studying Health Sciences at Maastricht University. As a part of this study, she fulfilled an internship at the Municipal Health Service (GGD) in Rotterdam in 2004. In 2005, she obtained her Master's degree in Health Sciences, specialization Environmental Health Science. From January through May 2005, she worked as employee environmental health at the Regional Health Service in Arnhem (Hulpverlening Gelderland Midden). In September 2005, she started studying for a Master's degree in Epidemiology at Maastricht University. At the Department of Epidemiology, Jessie fulfilled an internship on the topic of coffee and tea consumption in relation to ovarian cancer risk, and in September 2006, she graduated (cum laude). From August 2006 until August 2010, she worked on the PhD project entitled "Risk factors for Barrett's esophagus, adenocarcinoma of the esophagus and gastric cardia: a prospective cohort study", described in this thesis at the Department of Epidemiology, GROW - School for Oncology and Developmental Biology, Maastricht University Medical Centre +. Since October 2010 Jessie has worked at the Comprehensive Cancer Centre Limburg (IKL) as an epidemiologist. Jessie is living with her husband Joep Urlings.

LIST OF PUBLICATIONS

Submitted manuscripts

Steevens J, Schouten LJ, Goldbohm RA, Van den Brandt PA. Vegetables and fruits consumption and risk of esophageal and gastric cancer subtypes in the Netherlands Cohort Study.

Schouten LJ, Steevens J, Huysentruyt CJR, Coffeng C, Keulemans YCA, van Leeuwen FE, Driessen ALC, van den Brandt PA. Cancer incidence and total and cause-specific mortality in patients with Barrett's esophagus.

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