LIFESTYLE FACTORS AND RISK OF HEAD-NECK CANCER SUBTYPES:
a prospective cohort study

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Lifestyle factors and risk of head-neck cancer subtypes: a prospective cohort study

The research described in this thesis was conducted within the GROW - School for Oncology and Developmental Biology at the Department of Epidemiology, Maastricht University, in collaboration with the Department of Otorhinolaryngology, Head & Neck Surgery, Maastricht University Medical Center+, Maastricht, The Netherlands.

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Contents

Chapter 1
Introduction

This thesis concerns the associations between lifestyle factors and the risk of head-neck cancer (HNC) and HNC subtypes. In this chapter, we will first discuss the epidemiology and course of HNC. Subsequently, classification of HNC and HNC risk factors will be addressed. Finally, our study design and rationale, aim, and outline of the thesis will be considered.

Head-neck cancer

In this paragraph, we will discuss the epidemiology and course of HNC.

Incidence and mortality

HNC is one of the most frequently occurring cancers. It is the seventh most common type of cancer in the world and Europe, and includes, among others, malignancies originating from the oral cavity, pharynx, and larynx.[1,2] Worldwide, HNC represents about 6% of all cancer cases and accounts for an estimated 650,000 new cases and 350,000 cancer deaths every year.[1,3] More than 90% of all head and neck cancers are squamous cell carcinomas (SCC).[1] HNC is more frequent among men than women, especially for laryngeal cancer, and diagnosis of cancer of the mouth, pharynx, and larynx is most common in people aged 50 or over; the median age is around 60 years.[1] There is a slight decrease in the overall incidence of these three types of HNC in many high-income countries during the last decades, with exception of HPV-driven cancers of the oropharynx.

Course of the disease

Survival rates are variable and average around 50-60 per cent at 5 years.[1,4] However, the course of the disease largely depends on the type or anatomic sublocalization of HNC; survival is worse for specific primary sites such as the hypopharynx because of an often advanced stage at diagnosis. Treatment decisions are complex, due to different treatment options including surgery, radiation therapy, chemotherapy, and targeted therapy, and because of the necessity to spare or restore functional and cosmetic aspects.[1] However, these treatments often have considerable early and late side effects, resulting in significant morbidity with regard to basic functions such as speaking, swallowing, and breathing. In addition, there is a high recurrence risk in HNC, not only at the primary site, but also in the neck and at distance. Furthermore, the prognosis is influenced by the occurrence of second primary tumors, which are the result of so-called field cancerization of the head-neck area.[5]

It has been estimated that up to half of these cancers are preventable by associated lifestyle factors and appropriate diets.[4] Therefore, prevention appears to be a promising strategy in HNC, and insight into possible preventive lifestyle strategies is warranted.

Classification of head-neck cancer

The most frequently occurring subtypes of HNC are those originating from the oral cavity, pharynx, and larynx.[6] The pharynx can be further divided into naso-, oro-, and hypopharynx, but nasopharyngeal cancer is a rarity in the Netherlands and other western countries. HNC also includes malignancies from the salivary glands and paranasal sinuses, but these are far less common as well.[1]

HNC can be classified in several ways, depending on a more clinical or epidemiological point of view. From a clinical point of view, the TNM Classification of Malignant Tumours from the International Union Against Cancer (UICC)[7] is widely used. With regard to epidemiology and etiology, Hashibe et al.[8] from the International Head and Neck Cancer Epidemiology Consortium (INHANCE) classified HNC in their pooled analyses as oral cavity cancer (OCC), oro-/hypopharyngeal cancer (OHPC), and laryngeal cancer (LC) (Figure 1.1), according to the International Classification of Diseases for Oncology (ICD-O-3).[9] Both classifications are largely similar, with a classification dividing the oral cavity, pharynx, and larynx. However, in the TNM classification, the oro- and hypopharynx are distinguished and, in addition, the pharynx also includes the nasopharynx. As mentioned before, HNC located in the oral cavity, pharynx, and larynx are the most frequent HNC subtypes, and they share main risk factors.

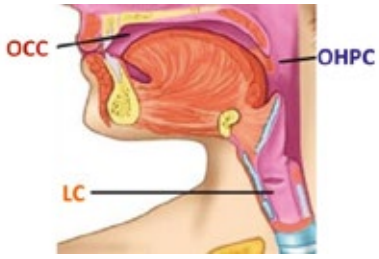

Figure 1.1. Classification of HNC subtypes.
(Adapted image from Google; search terms: 'respiratory system')

Relevance of head-neck cancer subtypes

It is relevant to distinguish the head-neck cancer subtypes mentioned above for several reasons. As stated before, treatment strategy and outcome differ per subtype, with generally the worst outcome for oro-/hypopharyngeal cancer. More importantly for the subject of this thesis, the main risk factors for HNC, like smoking, alcohol consumption, and dietary factors, are probably shared by all three subtypes, but the strengths of the associations differ. Therefore, it is relevant to classify HNC into OCC, OHPC, and LC when investigating these factors.

Risk factors for head-neck cancer

Several factors play a role in the development of HNC, which can be distinguished as demographic, lifestyle and dietary, and other factors.

Demographic factors

Increasing age and male sex are risk factors for developing HNC, as described above.[1,4] However, in the last decades, HNC incidence in relatively young adults has been on the rise, whereas the general incidence declined.[1,4] This rise in HNC incidence in young adults has been linked to HPV infection, which we will discuss later. Regarding sex, HNC is much more common among men, although this varies by subtype: OCC is also common in women, while OHPC and especially LC mostly occur in men.

Lifestyle and dietary factors

Alcohol consumption and cigarette smoking are the main risk factors for HNC originating from the oral cavity, pharynx, and larynx, and they are likely to be differentially associated with risk of those HNC subtypes.[4,10-14] Although cigarette smoking and alcohol consumption are clear risk factors for HNC, there are several remaining questions. It is not precisely clear whether a dose-response relationship[8,14] and the type of alcohol[15]—beer, wine or liquor—affect HNC risk, and what differential effect cigarette smoking and alcohol drinking have on HNC subtypes.[16] This has partly to do with the fact that the majority of conducted studies are case-control studies, a study design susceptible to selection and misclassification with regard to exposure. Prospective cohort studies are less sensitive to this bias, but only six population-based cohort studies have reported on alcohol consumption, cigarette smoking and HNC risk[17-23], often with a small number of cases and thereby hardly able to examine subtypes. Finally, a greater than multiplicative joint effect between alcohol and

tobacco consumption has been shown, but most evidence comes from case-control studies as well.[17,20-22,24-26]

Dietary factors probably influence HNC risk as well. In 2007, the World Cancer Research Fund (WCRF) concluded that consumption of non-starchy vegetables and fruits probably protects against HNC originating from the oral cavity, pharynx, and larynx.[4] However, the evidence was limited and mostly based on case-control studies. Only five population-based cohort studies investigated vegetable and fruit intake and HNC risk.[22,27-30] Most of them looked at few HNC cases[22,28,30], did not report on specific HNC subtypes[22,28] and combined HNC with esophageal[22,27,28] and gastric[30] cancer into upper aero-digestive tract cancer (UADTC) for analyses. In addition, adequate adjustment for confounding by smoking is vitally important since smokers are known to consume fewer fruits and vegetables[31], but the largest prospective study on this topic lacked information on smoking duration, an important aspect of smoking behavior.[18,29] The WCRF also concluded that foods containing carotenoids are probably protective against HNC, but mostly based on case-control studies, and that evidence regarding other micronutrients like vitamin C and E is scarce.[4] A protective effect of vitamin C and E is, however, plausible, given their antioxidant capacities. Only one prospective cohort study investigated the association between intake of (vitamins and) carotenoids and the risk on HNC.[23] Finally, selenium is an essential trace element present in food which may influence carcinogenesis particularly by its antioxidant properties.[32] In observational studies, selenium status has been associated with a decreased risk of prostate and esophageal cancer and possibly also of lung and stomach cancer.[4,33,34] A low selenium status may also be associated with an increased risk of HNC, but evidence regarding selenium and HNC risk is scarce and only based on case-control studies.[35-37]

Other factors

A low body mass index (BMI) has also been associated with HNC risk, but this association remains to be clarified. Regarding this topic, the WCRF concluded that data regarding the association between body fatness and HNC risk—based on case-control studies, which mostly found inverse associations—were insufficient to allow any conclusions to be drawn.[4] Three prospective cohort studies investigated the association between BMI and HNC risk, but showed mixed results.[38-40] It remains therefore unclear whether there is a true inverse association between BMI and HNC, or an association due to reverse causality, confounding, or effect modification.[4,41-43]

Finally, human papilloma virus (HPV) infection has been associated with risk of some types of HNC.[44-47] It is estimated that 30% of the oropharyngeal cancers are HPV-

positive and that within this group, the risk is especially increased for tonsil cancer and cancer of the base of the tongue. Risks for other subtypes (oral, other pharyngeal cancers, and laryngeal cancer) are moderately increased. The role of HPV in HNC carcinogenesis is mainly of importance in young HNC patients and has increased since 1990.[47-49]

Rationale and aim of the thesis

Since treatment options are limited and survival rates poor, the focus on prevention of HNC—by lifestyle—is of great importance. Given the limited evidence with regard to lifestyle and dietary factors and HNC risk, mainly based on case-control studies, it is crucial to confirm these associations in a prospective cohort study, with comprehensive adjustment for smoking and alcohol consumption. Therefore, the general aim of this thesis was to study and further establish the existing evidence regarding the association between several lifestyle factors and risk of developing HNC and HNC subtypes. We studied the association of 1) alcohol consumption and cigarette smoking; 2) consumption of vegetables and fruits; 3) intake of vitamins and carotenoids; 4) selenium status; and 5) BMI with HNC overall and HNC subtypes within the large prospective Netherlands Cohort Study (NLCS). We focused on the most frequent HNC subtypes[6] (those located in the oral cavity, pharynx, and larynx) and hypothesized that 1) the risk of HNC is higher in participants with a low intake of vegetables and fruits (and nutrients like carotenoids and vitamins), and those with low levels of toenail selenium; 2) associations of the studied risk factors with risk of HNC are modified by smoking and alcohol consumption; 3) risk of HNC is lower in participants with a high BMI; and that 4) these risks are different when studied for the HNC subtypes OCC, OHPC, and LC.

We believe our large prospective cohort study will add significantly to the current evidence. In addition, we hope to obtain a broader view regarding the development of HNC, with more insight into possible mechanisms regarding factors influencing HNC risk.

Study design

The NLCS was initiated in September 1986 and includes 120,852 participants, 58,279 men and 62,573 women, aged 55-69 years from 204 Dutch municipal population

registries.[50] At baseline, all participants completed a self-administered questionnaire on dietary habits and other cancer risk factors, such as smoking, alcohol consumption, and anthropometry. The 11-page questionnaire included a 150-item food frequency questionnaire that focused on habitual food consumption during the year preceding the start of the study. In addition, about 75% of the participants collected toenail clippings. The NLCS has been approved by the Medical Ethics Committee of Maastricht University (Maastricht, The Netherlands).

For data processing and analyses, the case-cohort design was used for efficiency in data processing and follow-up.[51] In this design, cases were derived from the entire cohort, whereas the number of person-years at risk for the entire cohort was estimated using a subcohort of 5,000 people, 2,411 men and 2,589 women. This subcohort was randomly sampled from the total cohort at baseline and followed up for vital status information. The entire cohort is being monitored for cancer incidence by annual record linkage to the Netherlands Cancer Registry (NCR), and the nationwide network and registry of histo- and cytopathology in the Netherlands (PALGA).[52]

Follow-up for vital status of the subcohort was nearly 100% complete after 20.3 years and the completeness of cancer follow-up is estimated to be ≥96%.[53] Since follow-up for the NLCS is currently ongoing, two different follow-up periods were used in this thesis, respectively 17.3 and 20.3 years. We excluded cohort members who reported having prevalent cancer other than skin cancer at baseline, as well as subjects with missing data on exposure and/or confounding variables. Participants with incomplete or inconsistent dietary data were also excluded from analyses regarding diet and anthropometry.[54,55] Only microscopically confirmed, first occurrences of squamous cell carcinomas were included.[1,4] After 20.3 years of follow-up, 415 HNC (131 OCC, 88 OHPC, and 193 LC) cases were available for analysis.

Outline of the thesis

This thesis starts with the study of alcohol consumption and cigarette smoking with regard to risk of HNC (and HNC subtypes) in our cohort (Chapter 2). In Chapter 3, the association between consumption of vegetables and fruits and the risk of HNC was investigated. Chapter 4 describes the relation between vitamin and carotenoid intake and HNC risk. Furthermore, the associations between toenail selenium status and BMI and HNC were studied in respectively Chapter 5 and 6. Finally, the findings described in this thesis are generally discussed and put into perspective in Chapter 7.

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Chapter 2

Alcohol consumption, cigarette smoking and the risk of subtypes of head-neck cancer: results from the Netherlands Cohort Study

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Abstract

Background

Prospective data on alcohol consumption, cigarette smoking and risk of head-neck cancer (HNC) subtypes, i.e., oral cavity cancer (OCC), oro-/hypopharyngeal cancer (OHPC), and laryngeal cancer (LC), are limited. We investigated these associations within the second largest prospective study on this topic so far, the Netherlands Cohort Study.

Methods

120,852 participants completed a questionnaire on diet and other cancer risk factors in 1986. After 17.3 years of follow-up, 395 HNC (110 OCC, 83 OHPC, and 199 LC) cases and 4,288 subcohort members were available for case-cohort analysis using Cox proportional hazards models.

Results

For total HNC, the multivariable-adjusted incidence rate ratio (RR) was 2.74 (95% confidence interval (CI) 1.85-4.06) for those drinking ≥30g ethanol/day compared with abstainers; in subtypes, RRs were 6.39 for OCC, 3.52 for OHPC, and 1.54 for LC. Compared with never cigarette smokers, current cigarette smokers had a RR of 4.49 (95% CI 3.11-6.48) for HNC overall, and 2.11 for OCC, 8.53 for OHPC, and 8.07 for LC. A significant, positive, multiplicative interaction between categories of alcohol consumption and cigarette smoking was found for HNC overall (*P* interaction=0.03).

Conclusions

Alcohol consumption and cigarette smoking were independently associated with risk of HNC overall, with a positive, multiplicative interaction. The strength of these associations differed among HNC subtypes: OCC was most strongly associated with alcohol consumption but most weakly with cigarette smoking, whereas LC was not statistically significantly associated with alcohol consumption.

Introduction

Head and neck cancer (HNC) includes several malignancies that originate in the paranasal sinuses, nasal cavity, salivary glands, oral cavity, pharynx, and larynx.[1] HNC is the seventh most common type of cancer in the world and in the European Union; in Europe, HNC accounts for an estimated 130,000 new cases every year.[2]

Alcohol consumption and cigarette smoking are established risk factors for HNC originating from the oral cavity, pharynx, and larynx, and are likely to be differentially associated with risk of those HNC subtypes.[3-8] However, the majority of conducted studies are case-control studies, a study design susceptible to misclassification with regard to exposure. Prospective cohort studies are less sensitive to this bias, but only six population-based cohort studies have reported on alcohol consumption, cigarette smoking and HNC risk.[9-15] Of these studies, most had a small number of cases and were thereby hardly able to examine subtypes; HNC was often combined with other cancers into upper aerodigestive tract cancer.[9,12-15] In addition, the largest prospective study so far lacked information on smoking duration.[10] Finally, a greater than multiplicative joint effect between alcohol and tobacco consumption has been shown, but most evidence comes from case-control studies as well.[9,12-14,16-18]

Therefore, we wanted to investigate these associations in HNC subtypes within the large prospective Netherlands Cohort Study (NLCS). We focused on the most frequent HNC subtypes: those located in the oral cavity, pharynx, and larynx, and hypothesized that 1) alcohol consumption and cigarette smoking are strongly, positively associated with HNC risk, with multiplicative interaction; and that 2) these risks are different for oral cavity cancer (OCC), oro-/hypopharyngeal cancer (OHPC), and laryngeal cancer (LC).

Methods

Study design and population

The present study was conducted within the NLCS, which started in September 1986 with the inclusion of 120,852 participants, aged 55-69 years from 204 Dutch municipal population registries.[19]

For data processing and analysis, the case-cohort design was used for reasons of efficiency.[20] Cases were derived from the total cohort, whereas the number of person-years at risk for the total cohort was estimated from a subcohort of 5,000 persons, randomly sampled from the entire cohort at baseline.

Follow-up for cancer incidence was done by annual record linkage to the Netherlands Cancer Registry and the nationwide network and pathology registry.[21] The completeness of cancer follow-up is estimated to be ≥96%[22], and follow-up for vital status of the subcohort was nearly 100% complete after 17.3 years.

We excluded cohort members who reported to have prevalent cancer other than skin cancer at baseline, and cases and subcohort members with missing data on exposure or confounding variables. Only microscopically confirmed, first occurrences of squamous cell carcinomas—which include nearly all malignancies of the mouth, pharynx, and larynx[1,3]—of the head and neck were included.

In total, 395 incident HNC cases and 4288 subcohort members were available for analysis (Figure 2.1). Of these cases, 110 were oral cavity cancer (ICD-O-3 C003-009, C020-C023, C030-C031, C039-C041, C048-C050, C060-C062, C068-C069), 83 oro-/hypopharyngeal cancer (C019, C024, C051-C052, C090-C091, C098-C104, C108-C109, C129-C132, C138-C139); 3 oral cavity, pharynx unspecified, or overlapping (C028-C029, C058-C059, C140-C142, C148), and 199 laryngeal cancer (C320-C329) cases, classified as proposed by Hashibe et al.[23], according to the International Classification of Diseases for Oncology (ICD-O-3).[24]

The NLCS has been approved by the Medical Ethics Committee of Maastricht University (Maastricht, The Netherlands).

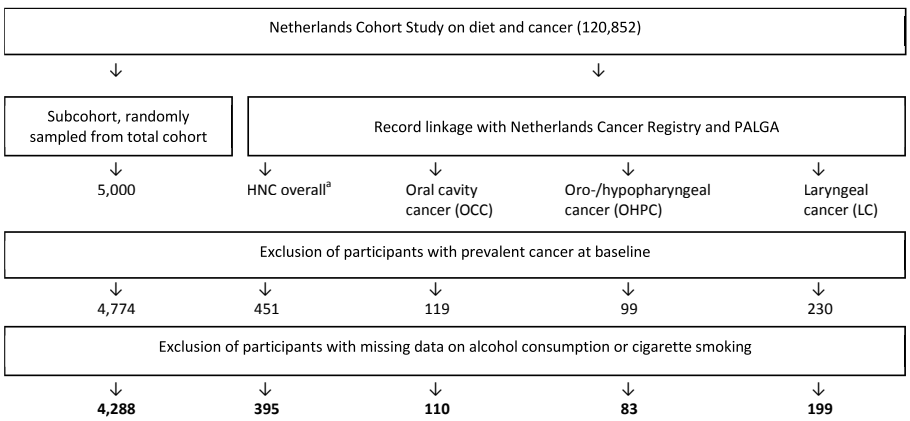

Figure 2.1. Flow diagram of the number of subcohort members and cases on whom the analyses were based.
Abbreviation PALGA: nationwide network and registry of histopathology and cytopathology in the Netherlands.
[a]Oral cavity cancer; oro-/hypopharyngeal cancer; oral cavity, pharynx unspecified or overlapping cancer; laryngeal cancer.

Exposure data

At baseline, all cohort members completed a self-administered questionnaire, which included a 150-item food frequency questionnaire (FFQ) with detailed questions on alcohol consumption, smoking habits, and other cancer risk factors.

We asked about the habitual intake of alcohol during the year preceding the start of the study, 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. In addition, 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'. Total mean daily ethanol intake was calculated using the Dutch food-composition table.[25] On the basis of pilot study data, standard glass sizes were defined as 200 mL for beer, 105 mL for wine, and 45 mL for liquor, corresponding to 8, 10, and 13 grams of ethanol, respectively.[26] We also asked questions about the consumption of 'beer' and 'other alcoholic beverages' 5 years before baseline and selected participants with stable alcohol consumption to perform a sensitivity analysis.[27] Participants who indicated that they used alcoholic beverages never or less than once a month were considered abstainers.

We asked detailed information regarding cigarette smoking. Among others, questions were asked about whether the subject was a smoker at baseline; age at which they started and stopped smoking; the number of cigarettes smoked daily and the number of smoking years (excluding stopping periods). Based on these questions, the following variables were constructed for analysis: smoking status (never/former/current); current smoking (yes/no); frequency (cigarettes/day); duration (years); the number of pack-years; and time since smoking cessation (years). We also asked about cigar and pipe smoking and the use of smokeless tobacco. Participants who indicated they had never smoked cigarettes were considered never smokers.

The FFQ was 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.[28] The reproducibility of the FFQ was assessed through annually repeated measurements in a subgroup of the subcohort and the test-retest correlation was 0.90 for alcohol intake; this correlation declined only 0.01-0.02 per year.[29]

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

Statistical analysis

Person-years at risk were calculated from baseline until diagnosis of HNC, death, emigration, loss to follow-up, or end of follow-up (i.e., 31 December 2003), whichever occurred first.

Age (years) and sex were considered predefined confounders. The potential confounders considered were[3,30,31]: level of education; non-occupational physical activity; energy intake; coffee and tea consumption; intake of fruit, vegetables, fish, fat, red meat, and meat products; and family history of head-neck cancer. Alcohol consumption and cigarette smoking were mutually adjusted in statistical models. A variable was considered a confounder if including it in the model changed the incidence rate ratio (RR) for HNC overall or any of the HNC subtypes by >10%; according to this, none of the potential confounders was included in the final model.

The Cox proportional hazards model was used to estimate incidence RRs and corresponding 95% confidence intervals (CI) for alcohol consumption and cigarette smoking in multivariable-adjusted case-cohort analyses. Analyses were done using the Stata 11.2 statistical software package (StataCorp, College Station, Texas, USA). Standard errors were calculated 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.[32] The proportional hazards assumption was assessed using the scaled Schoenfeld residuals.[33] If there was an indication for violation of the assumption for a variable, we further investigated this by adding a time-varying covariate for that variable to the model.

We also analyzed beer, wine, and liquor consumption, adjusted for ethanol intake, to examine whether substances in alcoholic beverages, other than ethanol, have an effect on HNC risk. In smoking analyses, different aspects of cigarette smoking were investigated and mutually adjusted for, in order to obtain a complete exposure model. The total number of cases that exclusively smoked cigar and/or pipe or used smokeless tobacco was too low (N<10) to further analyze associations with HNC risk.

When adjusting for smoking frequency, duration, or pack-years, we centered these continuous variables as proposed by Leffondré et al.[34]

Tests for linear dose-response trends were assessed by fitting ordinal exposure variables as continuous terms. To evaluate possible multiplicative interaction between categories of alcohol consumption and cigarette smoking, we estimated RRs of HNC overall and all HNC subtypes for combinations of these exposures. The interaction was investigated by including cross-product terms in the model and performing a Wald test. Two-sided P values are reported throughout the article.

Tests for heterogeneity among HNC subtypes were performed to investigate differences in associations among HNC subtypes by a bootstrapping method developed for the case-cohort design.[35] For each bootstrap sample, X subcohort members were randomly drawn from the subcohort of X subjects and Y cases from the total of Y cases outside the subcohort, both with replacement, out of the dataset of X + Y observations. The logRRs were obtained from this sample using Stata's competing risks procedure and recalculated for each bootstrap-replication. The confidence interval and *P* value of the differences in incidence rate ratio of the subtypes were then calculated from the replicated statistics. Each bootstrap analysis was based on at least 1,000 replications.[36]

Results

Compared to the subcohort, cases were more frequently men than women, and less often alcohol abstainers (Table 2.1). Among alcohol consumers, cases had a substantially higher alcohol intake and generally drank more beer, wine, and liquor than subcohort members. In both cases and subcohort members, men mostly consumed beer and liquor, whereas women drank more wine. With respect to cigarette smoking, cases were far more often current smokers and also smoked a substantially higher number of pack-years than subcohort members. Women were more often never smokers than men; among ever smokers, men generally smoked more pack-years than women, in cases and subcohort members.

Alcohol consumption

Alcohol consumption of ≥30 grams (g) per day compared with abstinence was associated with a statistically significantly increased risk of HNC overall (multivariable-adjusted RR: 2.74, 95% CI 1.85-4.06), OCC (RR: 6.39, 95% CI 3.13-13.03), and OHPC (RR: 3.52, 95% CI 1.69-7.36), but not LC (RR: 1.54, 95% CI 0.91-2.60) (Table 2.2). A strong dose-response relationship (*P* trend<0.001) was found between categories of increasing alcohol consumption and HNC overall, OCC, and OHPC risk. A significant interaction was found between sex and continuous alcohol consumption in HNC overall (*P*=0.02) and OCC (*P*=0.004), with women having higher RRs than men.

After adjustment for total alcohol intake, consumption of beer, wine, and liquor was generally not significantly associated with HNC risk. Beer consumption was, however, statistically significantly, positively associated with OHPC-risk (*P* trend=0.03); liquor consumption was significantly associated with an increased risk of OCC

Table 2.1. Characteristics of cases and subcohort members; Netherlands Cohort Study, 1986–2003

	Subcohort	Head-neck cancer cases			
		Overall	Subtypes		
			OCC[a]	OHPC[a]	LC[a]
Exposure variables and potential confounders[b]	**(*N*=4,288)[c]**	**(*N*=395)[c]**	**(*N*=110)[c]**	**(*N*=83)[c]**	**(*N*=199)[c]**
Age at baseline (years)	61.3 (4.2)	61.8 (4.1)	61.8 (4.3)	61.5 (4.2)	61.8 (4.0)
Sex: men (%)	49.2	79.5	59.1	73.5	94.0
Abstainer from alcohol (%)	23.9	12.4	10.9	13.3	13.1
Men (%)	14.8	9.2	4.6	9.8	10.7
Women (%)	32.6	24.7	20.0	22.7[d]	50.0[e]
Alcohol consumers:					
Ethanol intake (grams/day)	13.4 (15.0)	27.3 (25.6)	28.7 (25.4)	35.0 (31.6)	23.2 (22.1)
Men	17.5	29.1	34.3	40.2	23.5
Women	8.5	18.6	19.1	18.0	14.7
Beer intake (glasses/week)	2.2 (5.8)	6.3 (12.5)	4.4 (10.2)	10.3 (18.3)	5.8 (10.4)
Men	3.7	6.9	6.1	11.1	5.8
Women	0.3	3.5	1.4	7.9	4.5
Wine intake (glasses/week)	3.7 (5.6)	4.4 (9.1)	5.5 (8.1)	6.0 (11.5)	3.1 (8.4)
Men	3.1	4.0	4.2	6.7	3.1
Women	4.4	6.0	7.7	3.5	4.1
Liquor intake (glasses/week)	2.9 (5.7)	7.3 (10.0)	8.4 (11.2)	7.7 (11.7)	6.4 (8.3)
Men	4.6	8.2	11.5	9.4	6.6
Women	0.9	3.0	3.1	1.9	1.9
Cigarette smoking status (%)					
Total					
Never smokers	36.9	11.1	26.4	7.2	4.5
Former smokers	35.5	27.9	21.8	26.5	32.2
Current smokers	27.6	61.0	51.8	66.3	63.3

(continued) Exposure variables and potential confounders	Subcohort (*N*=4,288)	HNC overall (*N*=395)	OCC (*N*=110)	OHPC (*N*=83)	LC (*N*=199)
Cigarette smoking status (%)					
Men					
Never smokers	13.8	6.7	16.9	4.9	3.7
Former smokers	51.4	31.5	27.7	29.5	33.7
Current smokers	34.8	61.8	55.4	65.6	62.6
Women					
Never smokers	59.4	28.4	40.0	13.6[d]	16.7[e]
Former smokers	20.0	13.6	13.3	18.2	8.3
Current smokers	20.6	58.0	46.7	68.2	75.0
Ever cigarette smokers:					
Frequency of cigarette smoking (*N*/day)	15.3 (10.2)	19.5 (10.9)	19.9 (11.9)	21.4 (12.7)	18.6 (9.6)
Men	17.1	19.9	21.6	22.9	18.4
Women	11.6	17.4	16.5	16.7	22.0
Duration of cigarette smoking (years)	31.7 (12.1)	39.1 (9.5)	37.8 (9.2)	38.5 (9.8)	39.9 (9.4)
Men	33.6	39.8	38.9	39.8	40.0
Women	27.8	35.8	35.4	34.5	38.2
Pack-years of cigarette smoking (*N*)	22.8 (17.7)	34.4 (20.9)	34.5 (23.5)	37.0 (23.2)	33.5 (18.9)
Men	26.2	35.7	38.5	40.5	33.2
Women	16.0	28.3	26.4	26.3	38.5
Level of education (%)					
Primary	29.5	27.6	20.9	25.6	31.5
Lower vocational	22.0	18.9	17.3	17.1	20.8
Secondary and medium vocational	34.8	35.7	41.8	36.6	32.5
University and higher vocational	13.8	17.9	20.0	20.7	15.2

[a]OCC: oral cavity cancer; OHPC: oro-/hypopharyngeal cancer; LC: laryngeal cancer.

[b]Values are given as mean (SD); for categorical variables, *N* (%) is presented.

[c]The number of subcohort members or cases used in age- and sex-adjusted and multivariable-adjusted analyses of alcohol consumption and cigarette smoking.

[d]Based on only 22 female OHPC cases.

[e]Based on only 12 female LC cases.

Table 2.2. Multivariable-adjusted[a] associations between alcohol consumption and risk of head-neck cancer subtypes; Netherlands Cohort Study, 1986–2003

		Subcohort	Head-neck cancer cases								
			Overall		Subtypes						
					OCC[b]		OHPC[b]		LC[b]		
	Median	Person-time at risk (yrs)	No. cases	RR[b] (95% CI)	No. cases	RR (95% CI)	No. cases	RR (95% CI)	No. cases	RR (95% CI)	*P* hetero-geneity
Alcohol consumption (grams ethanol/day)											
Abstainers	0	15,255	49	1 (reference)	12	1 (reference)	11	1 (reference)	26	1 (reference)	0.86
>0 to <5	2	19,008	67	1.11 (0.75-1.65)	17	1.25 (0.59-2.65)	14	1.06 (0.47-2.40)	36	1.03 (0.60-1.77)	
5 to <15	9	14,468	72	1.15 (0.77-1.71)	19	1.91 (0.91-4.03)	12	0.90 (0.38-2.13)	40	0.94 (0.56-1.58)	
15 to <30	22	9,961	92	1.52 (1.02-2.27)	30	3.88 (1.86-8.12)	13	0.99 (0.41-2.38)	49	1.10 (0.66-1.83)	
≥30	40	5,659	115	2.74 (1.85-4.06)	32	6.39 (3.13-13.03)	33	3.52 (1.69-7.36)	48	1.54 (0.91-2.60)	
P for trend[c]				<0.001		<0.001		<0.001		0.05	
Continuous, 10 grams ethanol/day increment											
Overall		64,352	395	1.20 (1.12-1.27)	110	1.28 (1.18-1.39)	83	1.27 (1.16-1.38)	199	1.10 (1.02-1.18)	0.18
Men		30,169	314	1.19 (1.12-1.27)	65	1.27 (1.17-1.38)	61	1.27 (1.16-1.39)	187	1.10 (1.03-1.19)	
Women		34,183	81	1.40 (1.18-1.65)	45	1.58 (1.33-1.87)	22	1.31 (0.91-1.87)	12	0.85 (0.46-1.59)	
P for interaction[d]				0.02		0.004		0.68		0.67	
Alcohol consumption (grams ethanol/day) stable users[e]											
Abstainers	0	11,810	38	1 (reference)	9	1 (reference)	9	1 (reference)	20	1 (reference)	1.00
>0 to <5	2	11,813	36	0.98 (0.60-1.61)	12	1.65 (0.68-4.01)	7	0.86 (0.30-2.41)	17	0.72 (0.35-1.46)	
5 to <15	9	8,749	38	0.96 (0.58-1.59)	9	1.68 (0.63-4.47)	8	0.89 (0.32-2.47)	21	0.72 (0.37-1.40)	
15 to <30	22	5,293	45	1.27 (0.76-2.11)	12	3.20 (1.25-8.19)	6	0.72 (0.23-2.26)	27	0.96 (0.50-1.83)	
≥30	42	3,047	69	2.90 (1.78-4.73)	17	7.50 (3.15-17.88)	20	3.46 (1.46-8.20)	31	1.57 (0.82-3.02)	
P for trend				<0.001		<0.001		0.001		0.03	
Continuous, 10 grams ethanol/day increment		39,712	226	1.26 (1.16-1.36)	59	1.37 (1.24-1.52)	50	1.35 (1.20-1.52)	116	1.16 (1.04-1.28)	0.72

(continued)	Median	Person-time at risk (yrs)	No. cases	RR (95% CI)	No. cases	RR (95% CI)	No. cases	RR (95% CI)	No. cases	RR (95% CI)	*P* hetero-geneity
Alcoholic beverages (glasses/day)[f]											
Beer											
No beer	0	43,519	183	1 (reference)	59	1 (reference)	36	1 (reference)	87	1 (reference)	0.84
>0-<1	0.2	16,408	129	0.94 (0.71-1.24)	34	1.10 (0.65-1.86)	24	0.98 (0.54-1.76)	69	0.85 (0.60-1.22)	
1-<2	1.4	2,853	37	1.12 (0.72-1.74)	8	1.17 (0.49-2.77)	6	1.04 (0.41-2.66)	23	1.19 (0.71-2.01)	
≥2	3.4	1,554	46	1.39 (0.83-2.34)	9	0.99 (0.34-2.82)	17	2.48 (1.03-5.98)	20	1.30 (0.69-2.46)	
P for trend				0.14		0.95		0.03		0.20	
Cont., 1 glass/day incr.[f]		64,335	395	1.07 (0.97-1.19)	110	0.97 (0.80-1.16)[g]	83	1.19 (1.01-1.40)	199	1.08 (0.96-1.23)	0.07
Wine											
No wine	0	30,263	197	1 (reference)	44	1 (reference)	38	1 (reference)	114	1 (reference)	0.93
>0-<1	0.2	25,975	132	0.88 (0.67-1.14)	40	1.07 (0.67-1.71)	33	1.01 (0.59-1.75)	57	0.74 (0.52-1.05)	
1-<2	1.4	5,277	39	0.95 (0.63-1.44)	14	1.31 (0.67-2.55)	5	0.52 (0.19-1.39)	20	1.07 (0.63-1.83)	
≥2	2.6	2,751	24	0.56 (0.29-1.07)	11	0.93 (0.34-2.57)	7	0.52 (0.15-1.81)[g]	6	0.39 (0.15-0.99)	
P for trend				0.15		0.93		0.16		0.21	
Cont., 1 glass/day incr.		64,265	392	0.88 (0.74-1.05)	109	0.89 (0.69-1.16)	83	0.86 (0.64-1.17)	197	0.88 (0.68-1.14)	0.26
Liquor											
No liquor	0	33,299	137	1 (reference)	40	1 (reference)	34	1 (reference)	63	1 (reference)	0.44
>0-<1	0.2	23,492	133	1.09 (0.84-1.43)	31	1.10 (0.67-1.80)	23	0.86 (0.48-1.53)	78	1.17 (0.81-1.67)	
1-<2	1.9	5,370	67	1.09 (0.76-1.57)	18	1.65 (0.87-3.15)	12	0.79 (0.39-1.62)	37	1.08 (0.67-1.74)	
≥2	2.8	2,115	56	1.18 (0.71-1.95)	20	2.26 (1.02-4.99)	14	0.83 (0.33-2.13)	20	0.95 (0.47-1.93)	
P for trend				0.61		0.03		0.64		0.83	
Cont., 1 glass/day incr.		64 275	393	1.01 (0.86-1.18)	109	1.18 (0.89-1.56)	83	0.89 (0.68-1.15)	198	0.98 (0.80-1.21)	0.25

[a]Adjusted for age (years); sex; cigarette smoking (status (never/former/current), frequency (continuous; centered), and duration (continuous; centered)).

[b]OCC: oral cavity cancer; OHPC: oro-/hypopharyngeal cancer; LC: laryngeal cancer; RR: incidence rate ratio; CI: confidence interval.

[c]Tests for dose-response trends were assessed by fitting ordinal variables as continuous terms in the Cox proportional hazards model.

[d]*P* value for interaction between sex and alcohol consumption, based on cross-product terms in the Cox proportional hazards model and Wald test.

[e]Subjects who had not changed their continuous alcohol consumption habits in the 5 years before baseline: for "beer" and "other alcoholic beverages", participants could indicate whether 5 years before baseline they drunk (1) more than, (2) equal amounts of or (3) less than at baseline; the fourth answer option was (4) "I never use this".

[f]Additionally adjusted for continuous ethanol intake (g ethanol/day); cont.: continuous; incr.: increment.

[g]Proportional hazards assumption was possibly violated for the exposure variable, and there was a statistically significant interaction with time.

Table 2.3. Multivariable-adjusted[a] associations between cigarette smoking and risk of head-neck cancer subtypes; Netherlands Cohort Study, 1986–2003

		Subcohort	Head-neck cancer cases								
			Overall		Subtypes						
					OCC[b]		OHPC[b]		LC[b]		
	Median	Person-time at risk (yrs)	No. cases	RR[b] (95% CI)	No. cases	RR (95% CI)	No. cases	RR (95% CI)	No. cases	RR (95% CI)	*P* hetero-geneity
Cigarette smoking status											
Never smokers		25,051	44	1 (reference)	29	1 (reference)	6	1 (reference)	9	1 (reference)	0.97
Former smokers		22,644	110	1.44 (0.97-2.14)	24	0.70 (0.37-1.33)	22	2.68 (1.00-7.14)[i]	64	2.63 (1.26-5.47)	
Current smokers		16,657	241	4.49 (3.11-6.48)	57	2.11 (1.23-3.62)	55	8.53 (3.38-21.55)	126	8.07 (3.94-16.54)	
P for trend[c]				<0.001		0.001		<0.001		<0.001	
P for interaction with sex[d]				0.25		0.08		0.44		0.46	
Cigarette smoking status, additionally adjusted for frequency and duration of cigarette smoking[e]											
Never smokers		25,051	44	1 (reference)	29	1 (reference)	6	1 (reference)	9	1 (reference)	0.97
Former smokers		22,644	110	1.64 (1.08-2.49)	24	0.79 (0.40-1.58)	22	3.03 (1.09-8.45)	64	2.87 (1.34-6.13)	
Current smokers		16,657	241	3.51 (2.36-5.23)	57	1.91 (1.06-3.42)	55	7.49 (2.87-19.54)	126	5.26 (2.45-11.28)	
P for trend				<0.001		0.03		<0.001		<0.001	
Frequency of cigarette smoking (*N*/day)[f]											
Never smokers	0	25,051	44	1 (reference)	29	1 (reference)	6	1 (reference)	9	1 (reference)	0.99
>0 to <20	10	24,787	155	1.30 (0.84-2.01)	38	0.63 (0.30-1.32)	30	2.08 (0.73-5.94)	85	2.32 (1.07-5.04)	
≥20	20	14,514	196	2.23 (1.45-3.44)	43	1.06 (0.52-2.16)	47	4.67 (1.64-13.34)	105	3.75 (1.73-8.14)	
P for trend				<0.001		0.33		<0.001		<0.001	
Continuous, 10 cigarettes /day increment		64,352	395	1.25 (1.13-1.38)	110	1.20 (1.00-1.44)[j]	83	1.42 (1.20-1.69)	199	1.21 (1.08-1.36)	0.71
Duration of cigarette smoking (years)[g]											
Never smokers	0	25,051	44	1 (reference)	29	1 (reference)	6	1 (reference)	9	1 (reference)	<0.001
>0 to <20	13	7,433	20	1.00 (0.56-1.77)	4	0.38 (0.13-1.17)	5	2.11 (0.59-7.51)	11	1.88 (0.75-4.69)	
20 to <40	30	18,999	105	1.44 (0.95-2.21)	30	0.80 (0.41-1.59)	25	2.74 (0.98-7.68)	50	2.35 (1.09-5.06)	
≥40	43	12,868	226	2.45 (1.49-4.02)	47	0.98 (0.39-2.46)	47	3.89 (1.22-12.40)	129	4.81 (2.11-11.00)	
P for trend				<0.001		0.87		0.02		<0.001	
Continuous, 10 years incr.		64,352	395	1.28 (1.14-1.42)	110	1.03 (0.85-1.24)	83	1.36 (1.09-1.70)	199	1.49 (1.25-1.78)	0.25

(continued)		Subcohort	HNC overall		OCC		OHPC		LC		
	Median	Person-time at risk (yrs)	No. cases	RR (95% CI)	No. cases	RR (95% CI)	No. cases	RR (95% CI)	No. cases	RR (95% CI)	*P* hetero-geneity
Pack-years of cigarette smoking[h]											
Never smokers	0	25,051	44	1 (reference)	29	1 (reference)	6	1 (reference)	9	1 (reference)	1.00
>0 to <20	9	20,832	96	1.16 (0.77-1.76)	24	0.58 (0.30-1.14)	20	2.12 (0.77-5.80)	51	2.07 (0.97-4.41)	
20 to <40	28	12,732	132	1.65 (1.04- 2.60)	32	0.84 (0.39-1.83)	26	2.87 (0.94-8.79)	73	2.93 (1.33-6.48)	
≥40	48	5,736	123	2.82 (1.76-4.50)	25	1.28 (0.58-2.82)	31	6.49 (2.11-19.95)	66	4.79 (2.15-10.64)	
P for trend				<0.001		0.07		<0.001		<0.001	
Continuous, 10 pack-years increment		64,352	395	1.18 (1.11-1.25)	110	1.16 (1.04-1.28)	83	1.24 (1.12-1.36)	199	1.16 (1.09-1.24)	0.77
Cigarette smoking cessation (years)[i]											
Never smokers	25	25,051	44	1 (reference)	29	1 (reference)	6	1 (reference)	9	1 (reference)	<0.001
Quit ≥20	14	6,953	24	1.25 (0.72-2.19)	5	0.63 (0.22-1.81)	6	3.35 (0.97-11.55)	13	1.92 (0.79-4.70)	
Quit 10 to <20	5	7,717	36	1.49 (0.91-2.43)	8	0.78 (0.32-1.86)	8	3.29 (1.04-10.39)	20	2.45 (1.07-5.61)[k]	
Quit >0 to <10	0	7,918	50	1.73 (1.09-2.76)	11	0.84 (0.39-1.83)	8	2.48 (0.77-7.93)	31	3.45 (1.56-7.62)	
Current smokers		16,657	241	4.26 (2.93-6.20)	57	2.03 (1.16-3.56)	55	8.10 (3.14-20.87)	126	7.53 (3.65-15.51)	
P for trend				<0.001		0.004		<0.001		<0.001	

[a]All analyses were adjusted for age (years); sex; and alcohol consumption (grams ethanol/day; continuous).

[b]OCC: oral cavity cancer; OHPC: oro-/hypopharyngeal cancer; LC: laryngeal cancer; RR: incidence rate ratio; CI: confidence interval.

[c]Tests for dose-response trends were assessed by fitting ordinal variables as continuous terms in the Cox proportional hazards model.

[d]*P* value for interaction between sex and cigarette smoking status, based on cross-product terms in the Cox proportional hazards model and Wald test.

[e]Additionally adjusted for frequency (*N*/day; continuous; centered) and duration of cigarette smoking (years; continuous; centered).

[f]Analyses of cigarette smoking frequency were additionally adjusted for current cigarette smoking and duration of cigarette smoking (years; continuous; centered).

[g]Analyses of cigarette smoking duration were additionally adjusted for current cigarette smoking and frequency of cigarette smoking (*N*/day; continuous; centered); incr: increment.

[h]Analyses of cigarette smoking pack-years were additionally adjusted for current cigarette smoking.

[i]Cigarette smoking cessation was additionally adjusted for the no. of cigarette pack-years (continuous; centered).

[j]*P*<0.05.

[k]Proportional hazards assumption was possibly violated for the exposure variable, and there was a statistically significant interaction with time.

Table 2.4. Combinations of categories of alcohol consumption and frequency of cigarette smoking and risk (multivariable-adjusted[a] associations) of head-neck cancer subtypes; Netherlands Cohort Study, 1986–2003

	Head-neck cancer cases										
	Overall					Subtypes					
						OCC[b]		OHPC[b]		LC[b]	
	Alcohol consumption (grams ethanol/day)					Alcohol consumption[c]		Alcohol consumption[c]		Alcohol consumption[c]	
	0	>0 to <5	5 to <15	15 to <30	≥30	0-15	>15	0-15	>15	0-15	>15
Frequency of cigarette smoking (*N*/day)											
Never smokers											
Cases/PTAR[b]	10/8,959	13/9,729	7/4,245	11/1,499	3/619	21/22,933	8/2,118	3/22,933	3/2,118	6/22,933	3/2,118
RR	1 (ref)	1.20	1.23	5.53	2.97	1 (ref)	4.16	1 (ref)	10.18	1 (ref)	3.05
95% CI		0.52-2.75	0.46-3.29	2.27-13.49	0.78-11.40		1.82-9.52		2.03-51.06		0.72-12.92
>0 to <20											
Cases/PTAR	21/4,194	25/6,534	37/7,061	40/4,814	32/2,184	20/17,789	18/6,998	18/17,789	12/6,998	44/17,789	41/6,998
RR	1.89	1.56	2.04	2.63	3.81	0.76	1.55	4.02	5.63	2.66	4.19
95% CI	0.83-4.34	0.71-3.41	0.95-4.40	1.22-5.67	1.71-8.51	0.34-1.71	0.66-3.62	1.07-15.14	1.44-22.00	1.03-6.86	1.60-11.02
≥20											
Cases/PTAR	18/2,102	29/2,745	28/3,162	41/3,648	80/2,856	7/8,010	36/6,504	16/8,010	31/6,504	52/8,010	53/6,504
RR	2.78	3.88	2.85	3.32	8.28	0.58	3.54	7.26	16.12	5.42	5.54
95% CI	1.18-6.54	1.77-8.49	1.28-6.34	1.52-7.25	3.98-17.22	0.21-1.58	1.66-7.52	1.86-28.44	4.31-60.27	2.10-13.98	2.15-14.27
P for interaction[d]					0.03		0.10		0.09		0.19

[a]Adjusted for age; sex; current cigarette smoking; and duration of cigarette smoking (years; continuous; centered).

[b]OCC: oral cavity cancer; OHPC: oro-/hypopharyngeal cancer; LC: laryngeal cancer; PTAR: person-time at risk in years; RR: incidence rate ratio; CI: confidence interval.

[c]For the categorical interaction analyses in HNC-subtypes, it was necessary to aggregate categories of alcohol consumption (grams ethanol/day) in order to obtain sufficient numbers in strata.

[d]*P* value for interaction between categories of alcohol consumption and cigarette smoking, based on cross-product terms in the Cox proportional hazards model and Wald test.

(*P* trend=0.03). Wine consumption was largely inversely associated—although not statistically significantly—with risk of HNC overall and HNC subtypes.

Although RRs clearly varied among HNC subtypes, tests for heterogeneity did not show any significant differences in associations, possibly due to low power.

Cigarette smoking

Current cigarette smoking was statistically significantly associated with risk of HNC overall (multivariable-adjusted RR: 4.49, 95% CI 3.11-6.48) and all subtypes, with strongest associations in OHPC (RR: 8.53, 95% CI 3.38-21.55) and LC (RR: 8.07, 95% CI 3.94-16.54), compared with never smoking (Table 2.3). Compared with never smoking, former cigarette smoking was also associated with risk of HNC overall, although not statistically significantly (RR: 1.44, 95% CI 0.97-2.14), OHPC (RR: 2.68, 95% CI 1.00-7.14), and LC (RR: 2.63, 95% CI 1.26-5.47), but not OCC (RR: 0.70, 95% CI 0.37-1.33). Frequency and duration of cigarette smoking were also strongly, statistically significantly associated with an increased risk of HNC overall, OHPC, and LC (Table 2.3).

Regarding different aspects of cigarette smoking, after mutual adjustment, cigarette smoking status, frequency, and duration all remained statistically significantly associated with risk of HNC overall, OHPC, and LC (see Supplemental Tables 2.1 and 2.2). After additional adjustment for alcohol consumption (Table 2.3), most RRs between cigarette smoking status, frequency, duration and risk of HNC (subtypes) slightly attenuated, but remained statistically significantly associated with increased risks.

Results regarding smoking cessation show that the risk of HNC overall and all subtypes diminished for smokers who stopped smoking since <10, 10 to <20, or ≥20 years, compared with current smokers (all *P* trend<0.01) (Table 2.3). Nevertheless, compared with never smokers, RRs 20 years after smoking cessation were still elevated for HNC overall, OHPC, and LC, although not statistically significantly.

Despite considerable differences in RRs among HNC subtypes, tests for heterogeneity only showed statistically significant differences in associations for duration of cigarette smoking (*P*<0.001) and time since smoking cessation (*P*<0.001).

Interaction between alcohol consumption and cigarette smoking

For HNC overall, increased risks were found for every exposure combination of alcohol consumption and cigarette smoking, mostly statistically significantly, compared to never smokers and abstainers as reference group (Table 2.4). In addition, a statistically significant, positive, multiplicative interaction was found (*P* interaction=0.03) between categories of alcohol consumption and cigarette smoking, with a RR of 8.28 (95% CI

3.98-17.22), comparing participants smoking ≥ 20 cigarettes and drinking ≥30g alcohol per day with never smokers abstaining from alcohol.

In HNC subtypes, RRs were mostly increased as well when comparing participants smoking ≥ 20 cigarettes and drinking >15g alcohol per day with never smokers consuming 0 to 15g alcohol per day, with the highest RR for OHPC (RR: 16.12, 95% CI 4.31-60.27), but no significant interaction was found, possibly due to low numbers in strata.

Discussion

In this large prospective study on alcohol consumption, cigarette smoking, and risk of HNC (subtypes), alcohol consumption and cigarette smoking were strongly, independently associated with an increased risk of HNC overall. The strength of these associations however differed between HNC subtypes; OCC was most strongly associated with alcohol consumption but most weakly with cigarette smoking, whereas LC was not statistically significantly associated with alcohol consumption. For HNC overall, a multiplicative interaction between categories of alcohol consumption and cigarette smoking was found.

Alcohol consumption

Our results are in agreement with those of previous studies, showing alcohol consumption to be an independent risk factor for the development of HNC, with a strong, dose-response relationship.[4,9,11-14,17,23,37,38] Alcoholic beverages and acetaldehyde, the main metabolite of ethanol, are classified as a class I carcinogen.[18] It is plausible that alcohol—after being metabolized—acts both directly and indirectly in HNC carcinogenesis, the latter for example by acting as a solvent for other possible carcinogens, such as tobacco carcinogens.[3,39]

The differential risk among HNC subtypes is consistent with other studies, in which LC was also least associated with alcohol consumption.[8,40,41] However, several other studies found OHPC being most associated with alcohol consumption, although sometimes in specific subgroups, as opposed to OCC in our study.[11,23,41] Nevertheless, the differential risk among HNC subtypes is likely to be explained by the larynx having the least direct exposure to alcohol compared with the oral cavity and pharynx.[39,42] The slightly increased RRs for alcohol consumption and LC may be due to inhalation of alcohol containing aerosols, silent aspiration, systemic effects, and possibly residual confounding.

After adjustment for total alcohol intake, we generally found similar risks between intake of beer, wine, liquor and HNC. These findings imply that ethanol itself probably is the most important factor in determining HNC risk, rather than other substances in alcoholic beverages, which is in line with the results from other studies.[3,11,42] Consumption of wine was, however, generally inversely associated with HNC risk, as was also shown in a pooled analysis[42], which could be due to residual confounding by a general healthier lifestyle of wine-consumers in our study population.[3,42,43]

The significantly higher RRs between alcohol consumption and HNC risk in women as compared with men were seen earlier and could possibly be explained by women having stronger carcinogenic effects of alcohol at the same exposure level, suggesting possible gender-specific risk or protective factors.[11]

Cigarette smoking

This study confirms the strong associations of cigarette smoking with increased risk of HNC overall and all subtypes.[3,5,7,10,14,23,37,41] Among subtypes, however, OCC was least associated with cigarette smoking, and strongest associations were found with OHPC and LC. In addition, smoking status, frequency, and duration all appear to be of importance in the association between cigarette smoking and risk of HNC overall, OHPC, and LC. These results are generally consistent with previous reviews showing that cigarette smoking has a stronger effect on the larynx and/or pharynx than on the oral cavity[7,8,10,23,41]; in two meta-analyses, the larynx seemed to be clearly most susceptible to the effects of cigarette smoking.[23,41] A possible explanation for this could be the aerodynamics of respiratory flow in the upper airway: this flow changes from laminar in the oral cavity to turbulent in the larynx, which may result in the larynx and pharynx having a higher exposure to inhaled air—and thus to cigarette smoke—than the oral cavity.

Finally, our study shows smoking cessation leads to decreased HNC risks, which is in line with results from a recent pooled analysis as well.[44]

Interaction between alcohol consumption and cigarette smoking

Our study confirms a multiplicative interaction between categories of alcohol consumption and cigarette smoking in HNC overall.[9,14,16-18,37,38,41] The interaction effect between alcohol consumption and cigarette smoking is biologically plausible, since alcohol can act as a solvent for carcinogens in cigarette smoke and make the mucosa more permeable for these carcinogens; as a result, the carcinogenic properties of both factors are likely to be enhanced in the presence of one another.[3,39] Still, in HNC

subtypes, we had low numbers of cases in strata, which probably resulted in limited power to detect a significant deviation from the multiplicative model.

Strengths and limitations

Important strengths of our study are the prospective character and completeness of follow-up. Our study is the second largest prospective cohort study investigating alcohol consumption and cigarette smoking on the risk of HNC overall and subtypes so far.[9-15] Furthermore, we were able to take into account data on smoking duration, and to investigate as well as adjust for several aspects of smoking behavior.

A possible limitation of our study is the single measurement of exposure data. Alcohol consumption and cigarette smoking were however extensively addressed in the questionnaire, with questions about lifetime exposure history of smoking and alcohol intake 5 years before baseline. It is however possible that participants who smoked at baseline in 1986 stopped smoking at some point during follow-up or changed their alcohol intake, and this may have led to bias due to misclassification. Furthermore, although our study includes a large number of cases, a lack of power is a possible explanation for finding non-significant results for some associations and the tests for heterogeneity.

We lack information on human papillomavirus (HPV) infection. HPV infection is associated with HNC risk[45,46], but mainly with OHPC, in particular tonsil cancer and cancer of the base of the tongue. According to rates in our university medical center, only 25% of the diagnosed and treated oropharyngeal cancers between 1997 and 2003 were HPV-positive (all oropharyngeal cancer cases have been analyzed by p16-immunostaining and HPV16-specific fluorescence in situ hybridization (FISH), and—if FISH was negative—HPV-specific polymerase chain reaction). Moreover, the role of HPV in HNC carcinogenesis is mainly of importance in young HNC patients, and has increased since 1990.[47-49] Since our participants were aged 55-69 years at baseline in 1986, we assume that the number of HPV-associated HNC cases in our cohort is low, and we expect potential bias due to possible misclassification to be very limited.

Other factors we did not take into account in our analyses are the use of drugs and oral hygiene. Although we investigated several potential confounders, residual confounding is still possible, but we presume this to be limited as well.

It might also be interesting to examine the RRs of HNC for smokers among non-drinkers and for drinkers among non-smokers. However, as the case numbers for these subgroups would be too small to analyze, we decided not to investigate this.

Finally, though we wanted to examine the role of alcohol consumption and cigarette smoking in HNC subtypes, we did not investigate HNC located in the major

salivary glands, nasal cavity, paranasal sinuses, and nasopharynx, because of low numbers of these cases as well as a presumably different etiology.[50]

Conclusions

In conclusion, the present study, which is the second largest prospective cohort study regarding this topic so far, confirms the principal role of alcohol consumption and cigarette smoking in HNC carcinogenesis, as well as the differential associations with HNC subtypes, and a significant, positive, multiplicative interaction between both factors. As the existing evidence is largely based on case-control studies, this cohort study contributes to establish in which extent alcohol consumption and cigarette smoking are associated with risk of HNC overall and, more specifically, HNC subtypes.

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Supplemental Table 2.1. Age- and sex-adjusted associations[a] between cigarette smoking and risk of head-neck cancer subtypes; Netherlands Cohort Study, 1986–2003

		Subcohort	Head-neck cancer cases							
			Overall		Subtypes					
					OCC[b]		OHPC[b]		LC[b]	
	Median	Person-time at risk (yrs)	No. cases	RR[b] (95% CI)	No. of cases	RR (95% CI)	No. of cases	RR (95% CI)	No. of cases	RR (95% CI)
Cigarette smoking status										
Never smokers		25,051	44	1 (reference)	29	1 (reference)	6	1 (reference)	9	1 (reference)
Former smokers		22,644	110	1.57 (1.07-2.31)	24	0.77 (0.41-1.44)	22	2.91 (1.09-7.71)	64	2.78 (1.34-5.77)
Current smokers		16,657	241	5.35 (3.74-7.66)	57	2.73 (1.62-4.61)	55	10.88 (4.39-26.94)	126	8.86 (4.35-18.05)
P for trend[c]				<0.001		<0.001		<0.001		<0.001
Frequency of cigarette smoking (*N*/day)										
Never smokers	0	25,051	44	1 (reference)	29	1 (reference)	6	1 (reference)	9	1 (reference)
>0 to <20	10	24,787	155	2.44 (1.68-3.55)	38	1.27 (0.72-2.24)	30	4.42 (1.72-11.34)	85	4.09 (1.98-8.44)
≥20	20	14,514	196	4.76 (3.23-7.03)	43	2.48 (1.35-4.55)	47	11.42 (4.33-30.12)	105	7.13 (3.40-14.93)
P for trend				<0.001		0.002		<0.001		<0.001
Continuous, 10 cigarettes/ day increment		64,352	395	1.39 (1.29-1.50)	110	1.40 (1.22-1.61)	83	1.60 (1.41-1.82)	199	1.29 (1.19-1.41)
Duration of cigarette smoking (years)										
Never smokers	0	25,051	44	1 (reference)[d]	29	1 (reference)	6	1 (reference)	9	1 (reference)
>0 to <20	13	7,433	20	1.16 (0.67-2.01)	4	0.47 (0.16-1.38)	5	2.53 (0.75-8.55)	11	2.04 (0.82-5.07)
20 to <40	30	18,999	105	2.18 (1.46-3.26)	30	1.35 (0.74-2.46)	25	4.77 (1.79-12.69)	50	3.10 (1.45-6.61)
≥40	43	12,868	226	6.10 (4.14-9.00)	47	3.01 (1.65-5.50)	47	12.46 (4.77-32.53)	129	9.57 (4.56-20.08)
P for trend				<0.001		<0.001		<0.001		<0.001
Continuous, 10 years increment		64,352	395	1.60 (1.47-1.76)[e]	110	1.33 (1.15-1.53)[e]	83	1.80 (1.48-2.19)	199	1.81 (1.55-2.11)

[a]Analyses were adjusted for age (years) and sex, not for alcohol consumption; smoking aspects were also not mutually adjusted for.

[b]OCC: oral cavity cancer; OHPC: oro-/hypopharyngeal cancer; LC: laryngeal cancer; RR: incidence rate ratio; CI: confidence interval.

[c]Tests for dose-response trends were assessed by fitting ordinal variables as continuous terms in the Cox proportional hazards model.

[d]Proportional hazards assumption was possibly violated for the model, and there was a statistically significant interaction of the exposure variable with time.

[e]Proportional hazards assumption was possibly violated for the exposure variable, and there was a statistically significant interaction with time.

Supplemental Table 2.2. Multivariable-adjusted[a] associations between cigarette smoking and risk of head-neck cancer subtypes: (mutual) adjustment for smoking aspects; Netherlands Cohort Study, 1986–2003

		Subcohort	Head-neck cancer cases							
			Overall		Subtypes					
					OCC[b]		OHPC[b]		LC[b]	
	Median	Person-time at risk (yrs)	No. cases	RR[b] (95% CI)	No. of cases	RR (95% CI)	No. of cases	RR (95% CI)	No. of cases	RR (95% CI)
Cigarette smoking status, adjusted for frequency (*N*/day) and duration (years) of cigarette smoking										
Never smokers		25,051	44	1 (reference)	29	1 (reference)	6	1 (reference)	9	1 (reference)
Former smokers		22,644	110	1.78 (1.18-2.70)	24	0.86 (0.43-1.73)	22	3.22 (1.15-9.01)	64	3.01 (1.41-6.42)
Current smokers		16,657	241	4.05 (2.74-6.01)	57	2.40 (1.35-4.26)	55	9.18 (3.57-23.58)	126	5.62 (2.62-12.03)
P for trend[c]				<0.001		0.003		<0.001		<0.001
Frequency of cigarette smoking (*N*/day), adjusted for cigarette smoking status (non-current/current) and duration (years)										
Never smokers	0	25,051	44	1 (reference)	29	1 (reference)	6	1 (reference)	9	1 (reference)
>0 to <20	10	24,787	155	1.40 (0.91-2.15)	38	0.69 (0.33-1.44)	30	2.28 (0.80-6.45)	85	2.41 (1.11-5.22)
≥20	20	14,514	196	2.62 (1.72-4.00)	43	1.29 (0.64-2.59)	47	5.64 (1.99-15.97)	105	4.08 (1.89-8.80)
P for trend				<0.001		0.11		<0.001		<0.001
Continuous, 10 cig./day incr.		64352	395	1.34 (1.23-1.47)	110	1.32 (1.12-1.56)[d]	83	1.58 (1.36-1.83)	199	1.26 (1.12-1.40)
Duration of cigarette smoking (years), adjusted for cigarette smoking status (non-current/current) and frequency (*N*/day)										
Never smokers	0	25,051	44	1 (reference)	29	1 (reference)	6	1 (reference)	9	1 (reference)
>0 to <20	13	7,433	20	1.08 (0.61-1.90)	4	0.42 (0.14-1.29)	5	2.28 (0.64-8.06)	11	1.95 (0.78-4.88)
20 to <40	30	18,999	105	1.53 (1.00-2.33)[e]	30	0.85 (0.43-1.68)	25	2.80 (0.99-7.91)	50	2.44 (1.13-5.25)
≥40	43	12,868	226	2.78 (1.70-4.53)	47	1.18 (0.48-2.94)	47	4.54 (1.43-14.39)	129	5.12 (2.25-11.65)
P for trend				<0.001		0.62		0.009		<0.001
Continuous, 10 years incr.		64,352	395	1.30 (1.17-1.45)[f]	110	1.05 (0.87-1.27)	83	1.39 (1.11-1.74)	199	1.51 (1.26-1.80)

[a]Analyses were adjusted for age (years) and sex, not for alcohol consumption; analyses of cigarette smoking frequency and duration were adjusted for current cigarette smoking; cigarette smoking frequency and duration were also mutually adjusted for (continuous; centered) in analyses.

[b]OCC: oral cavity cancer; OHPC: oro-/hypopharyngeal cancer; LC: laryngeal cancer; RR: incidence rate ratio; CI: confidence interval; incr.: increment.

[c]Tests for dose-response trends were assessed by fitting ordinal variables as continuous terms in the Cox proportional hazards model.

[d]Proportional hazards assumption was possibly violated for the exposure variable, and there was a statistically significant interaction with time.

[e]*P*<0.05.

[f]Proportional hazards assumption was possibly violated for the model, and there was a statistically significant interaction of the exposure variable with time.

Chapter 3

Consumption of vegetables and fruits and risk of subtypes of head-neck cancer in the Netherlands Cohort Study

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Abstract

Background

There is limited prospective data on the relationship between consumption of vegetables and fruits and the risk of head-neck cancer (HNC) subtypes (i.e., oral cavity cancer (OCC), oro-/hypopharyngeal cancer (OHPC), and laryngeal cancer (LC)). Therefore, we investigated these associations within the Netherlands Cohort Study.

Methods

120,852 participants completed a 150-item food frequency questionnaire at baseline in 1986. After 20.3 years of follow-up, 415 cases of HNC (131 OCC, 88 OHPC, 3 oral cavity/pharynx unspecified or overlapping, and 193 LC) and 3,898 subcohort members were available for case-cohort analysis using Cox proportional hazards models.

Results

Total vegetable and fruit consumption was inversely associated with risk of HNC overall (multivariable-adjusted incidence rate ratio for highest vs. lowest quartile: 0.61, 95% confidence interval (CI) 0.44-0.85, *P* trend=0.002) and all HNC subtypes, with the strongest associations for OCC. Total vegetable intake and total fruit intake were also associated with a decreased risk of HNC overall and HNC subtypes. No significant interaction was found between vegetable and fruit intake and alcohol consumption or cigarette smoking.

Conclusions

In conclusion, in this large-scale cohort study, consumption of vegetables and fruits was associated with a decreased risk of HNC overall and all subtypes. Consumption of vegetables and fruits (or of specific groups of them) may protect against HNC and its subtypes.

Introduction

Head and neck cancer (HNC) is the seventh most common type of cancer in the world and Europe, and includes, among others, malignancies originating from the oral cavity, pharynx, and larynx.[1,2] Alcohol consumption, cigarette smoking and human papillomavirus (HPV) infection are established risk factors for HNC.[3,4] However, intake of vegetables and fruits has been consistently associated with a decreased risk of HNC.[3] Vegetables and fruits contain numerous substances, such as carotenoids and flavonoids, that may exert anticarcinogenic properties.[5]

In 2007, the World Cancer Research Fund concluded that consumption of non-starchy vegetables and fruits probably protects against HNC originating from the oral cavity, pharynx, and larynx.[3] However, the evidence was limited and is still mainly based on case-control studies, which are susceptible to bias with regard to exposure. In addition, adequate adjustment for confounding by smoking is vitally important since smokers are known to consume fewer fruits and vegetables.[6] For these reasons, it is crucial to confirm the inverse association between vegetable and fruit consumption and HNC risk in prospective cohort studies with comprehensive adjustment for smoking.

Only five population-based cohort studies have investigated vegetable and fruit intake and HNC risk.[7-11] Most of them looked at few HNC cases[8,10,11], did not report on specific HNC subtypes[8,11] (or only in very small numbers[10]), and combined HNC with esophageal[7,8,11] and gastric[10] cancer into upper aero-digestive tract cancer (UADTC) for analyses. Furthermore, the largest prospective study on this topic lacked information on smoking duration, an important aspect of smoking behavior.[9,12] Two prospective studies investigated possible effect modification by smoking and alcohol consumption on the link between vegetable and fruit intake and HNC risk, but they found no differential risk among strata.[7,9]

Therefore, we aimed to investigate the association between consumption of vegetables and fruits and HNC/HNC subtypes within the large prospective Netherlands Cohort Study (NLCS). We focused on the most frequent HNC subtypes[13] (those located in the oral cavity, pharynx, and larynx) and hypothesized that 1) the risk of HNC is higher in participants with low intake of vegetables and fruits; and that 2) these risks are different for oral cavity cancer (OCC), oro-/hypopharyngeal cancer (OHPC), and laryngeal cancer (LC). In addition, we will investigate the association of vegetables and fruits with HNC risk according to smoking status and categories of alcohol consumption.

Methods

Study design and population

The present study was conducted within the NLCS, which was initiated in September 1986 and includes 120,852 participants, aged 55-69 years from 204 Dutch municipal population registries.[14] At baseline, all participants completed a self-administered questionnaire about diet, lifestyle habits and other cancer risk factors. The NLCS has been approved by the Medical Ethics Committee of Maastricht University (Maastricht, The Netherlands).

We used the case-cohort design for efficiency in data processing and follow-up.[15] Cases were derived from the entire cohort. In contrast, the number of person-years at risk for the entire cohort was estimated using a subcohort of 5,000 people who were randomly sampled from the total cohort at baseline.

Follow-up for cancer incidence was done by record linkage to the Netherlands Cancer Registry (NCR) and the nationwide network and pathology registry (PALGA).[16] Follow-up for vital status of the subcohort was nearly 100% complete after 20.3 years and the completeness of cancer follow-up is estimated to be ≥96%.[17]

We excluded cohort members who reported having prevalent cancer other than skin cancer at baseline (Figure 3.1). Participants with incomplete or inconsistent dietary data and/or inconsistent vegetable data were also excluded from analysis.[18,19] Finally, subjects with missing data on confounding variables (300 subcohort members and 40 cases) were excluded. Only microscopically confirmed, first occurrences of squamous cell carcinomas—which include nearly all malignancies of the mouth, pharynx, and larynx—were included.[1,3]

In total, 3,898 subcohort members and 415 incident cases of the selected HNC subtypes were available for analysis (Figure 3.1). Of these cases, 131 were oral cavity cancer (ICD-O-3 C003-009, C020-C023, C030-C031, C039-C041, C048-C050, C060-C062, C068-C069), 88 were oro-/hypopharyngeal cancer (C019, C024, C051-C052, C090-C091, C098-C104, C108-C109, C129-C132, C138-C139), 3 were oral cavity/pharynx unspecified or overlapping (C028-C029, C058-C059, C140-C142, C148), and 193 were laryngeal (C320-C329) cancer cases. They were classified as proposed by Hashibe et al.[20] according to the International Classification of Diseases for Oncology (ICD-O-3).[21]

Questionnaire data

The questionnaire included a 150-item food frequency questionnaire (FFQ) that focused on habitual food consumption during the year preceding the start of the study. The FFQ covered most vegetables and fruits eaten regularly in 1986, with the

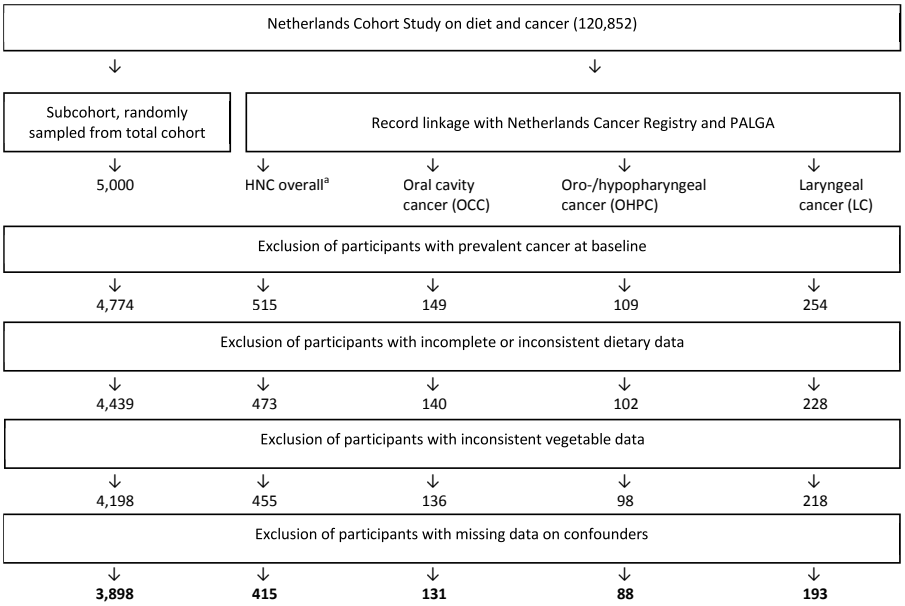

Figure 3.1. Flow diagram of the number of subcohort members and cases on whom the analyses were based.
Abbreviation PALGA: nationwide network and registry of histopathology and cytopathology in the Netherlands.
[a]Oral cavity cancer; oro-/hypopharyngeal cancer; oral cavity, pharynx unspecified or overlapping cancer; laryngeal cancer.

exception of chicory, cucumber and red cabbage. Broccoli was rarely available in the Netherlands in 1986 and was therefore not included in the FFQ.

In addition, we asked detailed questions about alcohol consumption and cigarette smoking. We asked about the habitual intake of alcohol during the year preceding the start of the study, measuring six items: 1) beer; 2) red wine; 3) white wine; 4) sherry and other fortified wines; 5) types of liquor that contained 16% alcohol on average; and 6) a category that included (Dutch) gin, brandy and whisky. In addition, we asked about the frequency of consumption and the number of glasses consumed on each drinking occasion. We also asked about the consumption of 'beer' and 'other alcoholic beverages' five years before baseline. Participants who indicated that they used alcoholic beverages never or less than once a month were considered to be abstainers. We also asked detailed questions about cigarette smoking, including whether the subject was a smoker at baseline, the age at which they started and stopped smoking, the number of cigarettes smoked daily, and the number of smoking years (excluding stopping periods).

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. In order to maintain blindness of data entry during follow-up, we selected an additional subcohort (from the total baseline cohort).

All subjects were asked to report their frequency of consumption of a number of vegetables (for summer and winter separately) and fruits (Table 3.1). Consumption frequency was specified by using categories ranging from 'never or less than once per month' to '3-7 times per week' for vegetable intake and '6-7 times per week' for fruit consumption. For individual fruit items, we also asked about the amount consumed per serving. In an open-ended question, participants could indicate which other vegetables and fruits they consumed on a regular basis, together with the consumption frequency (number of times per week) and amount consumed per serving. For onions and tomatoes, subjects were asked to report their usual intake in number per week; for sweet peppers, per month; and for mushrooms, the number of 250-gram boxes per month.

Participants were only asked about their usual serving size for string beans and cooked endive. Based on results of a pilot study, the mean of these serving sizes was used as a representative for the serving sizes of all solid and leafy vegetables, respectively. This individual serving size was then multiplied with a vegetable-specific factor to derive an individual serving size for each vegetable. To calculate mean daily vegetable consumption (grams/day) and the intake of individual fruit items (grams/day), we used consumption frequency and serving sizes.

Table 3.1. Composition of vegetable and fruit groups, based on vegetable and fruit items that were asked in the food frequency questionnaire

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 from an open-ended question on frequently consumed items not listed in the questionnaire
Brassica vegetables	Brussels sprouts, cabbage (white/green), cauliflower and kale
Leafy vegetables, cooked	Cooked endive and spinach
Leafy vegetables, raw	Lettuce and raw endive
Legumes	Broad beans, dried pulses and string beans
Allium vegetables	Leek and onions
Total fruits	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, and oranges and fresh orange juice

The FFQ was validated against a nine-day diet record. The Spearman correlation coefficient was 0.38 for total vegetable consumption and 0.60 for total fruit intake.[18] On average, vegetable consumption appeared to be slightly overestimated by the FFQ when compared to the diet records, whereas fruit consumption seemed to be underestimated.

The reproducibility of the FFQ was assessed by annual repeated measurements in a subgroup of the subcohort; the average test-retest correlation over all nutrients was 0.66 and ranged from 0.42 to 0.90.[22] The average decline in correlation amounted to 0.07 after five years, indicating that the ability of the FFQ to rank subjects according to nutrient intake maintains relatively well over time.[22]

Statistical analysis

The Cox proportional hazards model was used to estimate age- and sex-adjusted and multivariable-adjusted incidence rate ratios (RR) and corresponding 95% confidence intervals (CI). Person-years at risk were calculated from baseline until diagnosis of HNC, death, emigration, loss to follow-up, or end of follow-up, whichever occurred first. We analyzed total vegetable and fruit consumption, total vegetable consumption, consumption of cooked and raw vegetables, consumption of several vegetable subgroups and the most frequently consumed (<30% non-users in the subcohort) individual vegetables. In addition, we analyzed total fruit consumption, consumption of citrus fruits and the most frequently consumed individual fruits. The composition of each vegetable and fruit group can be found in Table 3.1. For categorical analyses, we categorized vegetable and fruit consumption levels into quartiles (Q) according to the sex-specific distribution in the subcohort; we chose an increment of 25 grams/day for continuous analyses.

The predefined confounders were age (years); sex; alcohol consumption (grams/day); and cigarette smoking (status (never/former/current), number of cigarettes smoked daily, and number of smoking years). Total vegetable and total fruit consumption were mutually adjusted in statistical models. We wanted to investigate whether vegetable and fruit subgroups and/or individual items have a specific additional effect on HNC risk, apart from being vegetables or fruits. Vegetables and fruits share some common characteristics and are, for example, generally low in calories and rich in fiber. However, other potential beneficial components (such as the amount and/or type of vitamins or phytochemicals) may vary among vegetable and fruit subgroups. In order to examine this possible additional effect, analyses of vegetable/fruit subgroups and individual items were adjusted for total vegetable and fruit intake.

We considered the following potential confounders: level of education; body mass index; non-occupational physical activity; intake of fish, red meat, and meat products (all grams/day); and family history of HNC.[3,23] A variable was regarded as a confounder if including it in the model changed the RR for intake of total vegetables and fruits (continuous) for HNC overall or any of the HNC subtypes by >10%. According to this, only the predefined confounders were included in the final model. When adjusting for cigarette smoking frequency and duration, we centered these continuous variables as proposed by Leffondré et al.[24]

We assessed tests for linear dose-response trends by fitting ordinal exposure variables as continuous terms. Standard errors were estimated using the robust Huber-White sandwich estimator to account for additional variance due to sampling from the cohort; this method is equivalent to Barlow's[25] variance-covariance estimator. The proportional hazards (PH) assumption was assessed using the scaled Schoenfeld residuals.[26] If there was an indication for violation of the assumption for a variable, it was further investigated by adding a time-varying covariate for that variable to the model.

We conducted analyses of total vegetable and fruit consumption stratified by sex in order to evaluate possible interaction. No statistically significant interaction with sex was found (*P* interaction for HNC overall=0.43); therefore, all analyses were carried out for both sexes combined. To determine whether alcohol consumption and/or cigarette smoking modify the association of total vegetable and fruit intake with HNC risk, we performed analyses in strata of alcohol consumption (abstainers; consuming >0 to 15 grams ethanol/day; consuming ≥15 grams ethanol/day) and cigarette smoking status (never/former/current). Alcohol consumption and cigarette smoking were mutually adjusted in these models. *P* values for interaction were assessed by including cross-product terms in the models and performing a Wald test.

To prevent bias caused by reversed causation, we checked for possible influence of preclinical cancer at baseline for consumption of vegetables and fruits. First, we compared the median vegetable and fruit consumption of cases diagnosed during the first two years of follow-up with the consumption of cases diagnosed later in follow-up. HNC cases diagnosed after the second year of follow-up (*N*=382) had a median consumption of 173 grams of vegetables and 109 grams of fruit per day. Cases diagnosed during the first two years of follow-up (*N*=33) had a daily median consumption of 176 grams of vegetables and 91 grams of fruit.

Second, after we applied a square-root transformation to normalize the distribution of the vegetable and fruit variables, we used an independent samples t-test to test the statistical significance of differences; there was no statistically

significant difference in vegetable (*P*=0.65) or fruit (*P*=0.36) consumption between early and late cases. Based on these results, we decided to include the total follow-up time in our analyses. In addition, we performed sensitivity analyses regarding the association between consumption of total vegetables and fruits, total vegetables, and total fruits, and the risk of HNC overall. We found essentially similar results for the total follow-up period and the period with exclusion of the first two years of follow-up (data not shown).

For HNC overall, the PH assumption was violated for current smoking—and a time-varying covariate showed a statistically significant interaction with time—in all categorical and continuous analyses. Although smoking was extensively addressed in the questionnaire (with questions about lifetime exposure history), we lack data after baseline. It is possible that participants who did smoke at baseline stopped smoking at some point during follow-up, which may explain this interaction with time. We did not find this for HNC subtypes, possibly as a result of less power. However, it seems theoretically plausible that the interaction of current smoking with time is also present in OCC, OHPC, and LC. Therefore, we decided to perform analyses for HNC and all HNC subtypes using a time-varying covariate for current smoking.

All reported *P* values were based on two-sided tests and considered statistically significant if <0.05. Analyses were done using the Stata 11.2 statistical software package (StataCorp, College Station, Texas, USA).

Results

Baseline characteristics

After 20.3 years of follow-up, 415 cases of HNC overall (131 OCC, 88 OHPC, 3 oral cavity/pharynx unspecified or overlapping, and 193 LC) were available for analysis (Figure 3.1), with a mean follow-up of 16.9 years and a total of 65,980 person-years at risk in the subcohort. Characteristics of our study population are shown in Table 3.2. Daily median total vegetable and fruit consumption was considerably lower among cases (292 grams for HNC overall) than subcohort members (344 grams), almost entirely the result of lower fruit consumption among cases. Compared to the subcohort, cases were more often men than women, especially LC cases. Among subcohort members and cases, women consumed substantially more fruit than men. Furthermore, there were far more current smokers among cases than subcohort members; in addition, cases smoked for a substantially higher number of pack-years.

Table 3.2. Characteristics of cases and subcohort members; Netherlands Cohort Study, 1986–2006

	Subcohort	Head-neck cancer cases			
		Overall	Subtypes		
			OCC[a]	OHPC[a]	LC[a]
Exposure variables and potential confounders[b]	**(*N*=3,898)[c]**	**(*N*=415)[c]**	**(*N*=131)[c]**	**(*N*=88)[c]**	**(*N*=193)[c]**
Age at baseline (years)	61.3 (4.2)	61.7 (4.1)	62.0 (4.2)	61.6 (4.0)	61.5 (4.0)
Sex: men (%)	49.3	77.1	57.3	73.9	92.8
Total vegetable and fruit consumption (g/day)	344 (258-446)	292 (211-400)	306 (225-396)	266 (194-382)	294 (220-407)
Men	320 (242-417)	286 (205-388)	298 (210-387)	259 (190-372)	293 (215-405)
Women	368 (282-467)	339 (245-435)	348 (244-419)	331 (245-465)	353 (253-485)
Total vegetable consumption (g/day)	179 (137-229)	175 (126-223)	179 (125-227)	171 (126-218)	174 (130-224)
Men	176 (135-227)	174 (128-219)	180 (130-235)	160 (122-202)	175 (130-224)
Women	181 (140-230)	175 (126-235)	175 (125-225)	196 (141-248)	168 (127-242)
Cooked vegetables (g/day)	143 (108-183)	138 (102-183)	136 (103-186)	137 (103-175)	143 (102-185)
Raw vegetables (g/day)	34 (20-53)	30 (17-50)	29 (19-50)	30 (19-59)	30 (15-48)
Leafy vegetables, cooked (g/day)	19 (12-29)	19 (12-30)	18 (11-31)	17 (12-27)	20 (12-31)
Leafy vegetables, raw (g/day)	8 (4-14)	7 (4-13)	8 (4-13)	7 (4-13)	7 (4-13)
Brassica vegetables (g/day)	28 (18-42)	30 (18-42)	26 (18-41)	31 (18-40)	32 (19-46)
Allium vegetables (g/day)	28 (16-44)	28 (16-44)	27 (17-48)	29 (11-45)	27 (16-43)
Legumes (g/day)	28 (18-42)	30 (18-44)	30 (17-48)	30 (19-43)	31 (19-42)
Total fruit consumption (g/day)	156 (95-234)	113 (56-203)	117 (65-192)	99 (36-196)	115 (58-214)
Men	137 (79-210)	103 (52-194)	109 (49-182)	81 (38-170)	111 (57-208)
Women	176 (112-255)	141 (67-238)	124 (74-225)	165 (31-239)	179 (73-305)
Citrus fruits (g/day)	56 (19-93)	32 (10-83)	40 (9-84)	40 (10-117)	28 (11-80)
Cigarette smoking status (%)					
Never smoker	36.8	13.5	29.0	8.0	5.7
Former smoker	36.4	30.1	25.2	29.6	34.2
Current smoker	26.8	56.4	45.8	62.5	60.1

(continued)	**Subcohort**	**Head-neck cancer cases**			
		Overall	**Subtypes**		
			OCC	**OHPC**	**LC**
Exposure variables and potential confounders	**(*N*=3,898)**	**(*N*=415)**	**(*N*=131)**	**(*N*=88)**	**(*N*=193)**
Ever cigarette smokers:					
Frequency of cigarette smoking (*N*/day)	15.3 (10.2)	19.5 (11.0)	20.4 (11.9)	21.1 (12.7)	18.4 (9.5)
Duration of cigarette smoking (years)	31.6 (12.2)	38.6 (9.9)	36.8 (10.1)	38.0 (10.3)	39.7 (9.6)
Pack-years of cigarette smoking (*N*)	22.7 (17.6)	34.1 (21.3)	34.9 (23.6)	36.2 (23.5)	32.8 (18.9)
Abstainer from alcohol (%)	23.6	9.9	8.4	12.5	9.8
Alcohol consumers: ethanol intake (g/day)	13.6 (15.1)	26.9 (26.2)	26.7 (27.3)	34.9 (31.4)	23.5 (22.1)
Level of education (%)					
Primary (men)	27.9 (23.4)	28.2 (27.7)	22.1 (17.3)	23.0 (21.5)	34.0 (34.5)
Lower vocational (men)	21.5 (20.0)	17.5 (16.4)	16.8 (13.3)	17.2 (16.9)	18.3 (17.5)
Secondary and medium vocational (men)	36.1 (36.8)	35.2 (34.9)	39.7 (42.7)	37.9 (33.9)	31.4 (32.2)
University and higher vocational (men)	14.6 (19.8)	19.2 (21.1)	21.4 (26.7)	21.8 (27.7)	16.2 (15.8)
Body mass index (kg/m²)	25.0 (3.1)	24.8 (2.7)	25. (3.0)	24.4 (2.6)	25.0 (2.6)
Non-occupational physical activity (min/day)	73 (60)	71 (58)	67 (60)	66 (55)	76 (58)
Energy intake (kJ/day)					
Men	9,047 (2,085)	9,070 (2,090)	8,712 (2,332)	9,071 (1,805)	9,235 (2,066)
Women	7,058 (1,632)	6,810 (1,940)	6,639 (2,052)	7,153 (1,607)	6,992 (1,841)
Fish consumption (g/day)	14 (7-22)	16 (8-23)	16 (7-22)	20 (12-26)	15 (7-22)
Red meat consumption (g/day)	85 (63-108)	86 (66-112)	83 (61-106)	84 (54-112)	91 (71-117)
Meat product consumption (g/day)	12 (5-21)	14 (7-24)	12 (6-21)	14 (7-21)	15 (7-26)
First-grade family history of HNC (%)	2.0	1.7	1.5	1.1	1.6

[a]OCC: oral cavity cancer; OHPC: oro-/hypopharyngeal cancer; LC: laryngeal cancer.

[b]Values are given as mean (SD); for categorical variables, *N* (%) is presented. For dietary variables, medians and interquartile ranges (P25-P75) are shown because of their right-skewed distribution.

[c]The number of subcohort members or cases used in age- and sex-adjusted and multivariable-adjusted analyses of vegetable and fruit consumption.

Cases were also less frequently alcohol abstainers and, among alcohol consumers, cases had a considerably higher alcohol intake than subcohort members.

The intake of specific items of vegetables and fruits of HNC overall cases and subcohort members is presented in Supplemental Table 3.1; individual vegetable and fruit items are ranked according to the percentage of non-users in the subcohort. Of the items asked about in the questionnaire, string beans, cauliflower and lettuce were the most frequently eaten vegetables in our study population, while raw carrots, gherkins and vegetable juices were consumed the least. Tomatoes, onions and string beans were the vegetables eaten in the largest amounts. The most consumed fruits in the subcohort were apples and pears, strawberries, and oranges; apples and pears and oranges were also the fruits consumed in the largest amounts.

Main analyses

Results of the multivariable-adjusted analyses on total vegetable and fruit consumption and vegetable and fruit subgroups are shown in Table 3.3. Results from age- and sex-adjusted analyses (data not shown) were largely comparable to the multivariable-adjusted results, but with most (inverse) associations between vegetable and fruit consumption and HNC risk being stronger in age- and sex-adjusted analyses.

Total vegetable and fruit consumption was associated with a decreased risk of HNC overall and of all HNC subtypes, with statistically significant associations in HNC overall (multivariable-adjusted RR for Q4 vs. Q1: 0.61, 95% CI 0.44-0.85, *P* trend=0.002), OCC (RR: 0.46, 95% CI 0.27-0.81, *P* trend=0.005) and OHPC (RR: 0.51, 95% CI 0.26-1.00, *P* trend=0.03) (Table 3.3). For LC, we found inverse (but not statistically significant) associations as well (RR for Q4 vs. Q1: 0.80, 95% CI 0.51-1.23, *P* trend=0.32). Total vegetable intake was also consistently inversely associated with HNC risk, but only statistically significantly with HNC overall (RR for Q4 vs. Q1: 0.71, 95% CI 0.51-0.99, *P* trend=0.07) (Table 3.3). When investigating the effect of specific groups of vegetables on HNC risk, we found that raw leafy vegetables statistically significantly decreased the risk of HNC overall (RR for Q4 vs. Q1: 0.67, 95% CI 0.47-0.96, *P* trend=0.05), adjusted for total vegetable and fruit intake. Among HNC subtypes, raw leafy vegetables were generally associated with decreased risks as well, but only with statistical significance in OHPC (RR for Q4 vs. Q1: 0.46, 95% CI 0.23-0.93, *P* trend=0.11). Point estimates (Q4 vs. Q1 or per 25g/day increment) for other vegetable subgroups varied below and above unity, and were not statistically significant (neither for HNC overall nor for HNC subtypes).

Table 3.3. Multivariable-adjusted[a] associations between vegetable and fruit consumption and risk of head-neck cancer subtypes; Netherlands Cohort Study, 1986–2006

			Subcohort	Head-neck cancer cases							
				Overall		Subtypes					
						OCC[b]		OHPC[b]		LC[b]	
	Median (g/day)		Person-time	No.	RR[c] (95% CI)	No.	RR (95% CI)	No.	RR (95% CI)	No.	RR (95% CI)
	Men	*Women*	at risk (yrs)	cases		cases		cases		cases	
Total vegetables and fruits											
Q1	188	227	16,279	150	1 (reference)	47	1 (reference)	37	1 (reference)	64	1 (reference)
Q2	282	325	16,538	99	0.73 (0.55-0.98)	33	0.75 (0.47-1.19)	21	0.64 (0.36-1.13)	44	0.77 (0.50-1.17)
Q3	364	412	16,455	86	0.62 (0.45-0.85)	29	0.63 (0.38-1.04)	13	0.38 (0.19-0.74)	44	0.77 (0.50-1.17)
Q4	496	552	16,708	80	0.61 (0.44-0.85)	22	0.46 (0.27-0.81)	17	0.51 (0.26-1.00)[h]	41	0.80 (0.51-1.23)
P for trend[d]					0.002		0.005		0.03		0.32
Continuous, per 25 g/day incr.			65,980	415	0.97 (0.95-0.99)	131	0.95 (0.92-0.99)	88	0.96 (0.90-1.01)[i]	193	0.99 (0.96-1.02)
Total vegetables[e]											
Q1	109	113	16,130	121	1 (reference)	39	1 (reference)	26	1 (reference)	54	1 (reference)
Q2	156	161	16,661	97	0.79 (0.58-1.07)	27	0.68 (0.41-1.13)	21	0.77 (0.42-1.42)	49	0.91 (0.60-1.38)
Q3	199	203	16,678	100	0.83 (0.61-1.13)	33	0.86 (0.52-1.41)	23	0.86 (0.47-1.59)	43	0.81 (0.52-1.24)
Q4	271	277	16,511	97	0.71 (0.51-0.99)	32	0.71 (0.41-1.24)	18	0.52 (0.24-1.10)	47	0.83 (0.53-1.29)
P for trend					0.07		0.36		0.10		0.39
Continuous, per 25 g/day incr.			65,980	415	0.96 (0.92-1.01)	131	0.95 (0.89-1.02)	88	0.94 (0.85-1.04)	193	0.98 (0.92-1.04)
Cooked vegetables[f]											
Q1	85	87	16,295	119	1 (reference)	38	1 (reference)	23	1 (reference)	56	1 (reference)
Q2	125	124	16,478	100	0.95 (0.69-1.30)	32	1.00 (0.59-1.67)	28	1.40 (0.78-2.54)	40	0.76 (0.48-1.18)
Q3	160	159	16,824	96	0.96 (0.68-1.34)	28	0.94 (0.53-1.66)	19	0.98 (0.48-1.98)	48	0.94 (0.60-1.48)
Q4	219	216	16,383	100	0.99 (0.67-1.48)	33	1.17 (0.59-2.30)	18	0.82 (0.33-2.03)	49	0.96 (0.57-1.61)[i]
P for trend					0.99		0.70		0.51		0.93
Continuous, per 25 g/day incr.			65,980	415	0.99 (0.93-1.05)	131	1.01 (0.91-1.13)	88	0.94 (0.82-1.08)	193	0.99 (0.92-1.07)
Raw vegetables[f]											
Q1	8	11	16,158	122	1 (reference)	36	1 (reference)	27	1 (reference)	58	1 (reference)
Q2	24	29	16,623	107	1.03 (0.76-1.41)	38	1.24 (0.75-2.06)	23	1.07 (0.57-2.00)	46	0.93 (0.60-1.42)
Q3	40	45	16,606	87	0.82 (0.58-1.14)	27	0.90 (0.51-1.58)	15	0.68 (0.33-1.42)	44	0.84 (0.54-1.32)
Q4	67	72	16,594	99	0.92 (0.64-1.32)	30	0.96 (0.51-1.79)	23	0.97 (0.47-2.00)	45	0.87 (0.55-1.40)
P for trend					0.48		0.68		0.77		0.54

(continued)	Median (g/day)		Subcohort	HNC overall		OCC		OHPC		LC	
	Men	*Women*	Person-time at risk (yrs)	No. cases	RR (95% CI)	No. cases	RR (95% CI)	No. cases	RR (95% CI)	No. cases	RR (95% CI)
Continuous, per 25 g/day incr.			65,980	415	0.99 (0.87-1.13)	131	0.94 (0.77-1.15)	88	1.10 (0.84-1.44)	193	0.97 (0.82-1.17)
Leafy vegetables, cooked[f]											
Q1	5	5	16,514	107	1 (reference)	36	1 (reference)	24	1 (reference)	46	1 (reference)
Q2	15	15	16,443	105	1.01 (0.74-1.39)	33	0.94 (0.57-1.56)	28	1.18 (0.66-2.13)	43	0.96 (0.61-1.50)
Q3	23	24	16,574	96	1.07 (0.78-1.47)	28	0.93 (0.55-1.59)	18	0.87 (0.46-1.66)[j]	49	1.23 (0.79-1.92)
Q4	39	38	16,450	107	1.17 (0.84-1.62)	34	1.19 (0.70-2.01)	18	0.87 (0.45-1.67)	55	1.28 (0.82-2.00)
P for trend					0.33		0.52		0.48		0.18
Continuous, per 25 g/day incr.			65,980	415	1.08 (0.90-1.30)	131	1.18 (0.88-1.58)	88	0.95 (0.66-1.37)	193	1.08 (0.85-1.38)
Leafy vegetables, raw[f]											
Q1	1	1	12,592	102	1 (reference)	26	1 (reference)	28	1 (reference)	46	1 (reference)
Q2	4	4	14,819	89	0.79 (0.57-1.10)	32	1.15 (0.66-1.99)	15	0.48 (0.25-0.94)	42	0.82 (0.52-1.29)
Q3	9	9	21,307	139	0.84 (0.62-1.15)[i]	46	1.14 (0.68-1.94)	28	0.60 (0.33-1.09)	64	0.86 (0.56-1.30)
Q4	20	20	17,263	85	0.67 (0.47-0.96)	27	0.88 (0.48-1.62)	17	0.46 (0.23-0.93)	41	0.74 (0.46-1.19)
P for trend					0.05		0.48		0.11		0.29
Continuous, per 25 g/day incr.			65,980	415	0.66 (0.45-0.95)	131	0.81 (0.45-1.45)	88	0.53 (0.21-1.34)	193	0.66 (0.40-1.07)
Brassica vegetables[f]											
Q1	12	12	16,181	117	1 (reference)	39	1 (reference)	26	1 (reference)	51	1 (reference)
Q2	24	23	16,542	83	0.70 (0.50-0.97)	34	0.87 (0.53-1.45)	15	0.56 (0.28-1.13)	32	0.60 (0.38-0.97)
Q3	35	34	16,841	111	0.99 (0.73-1.36)	29	0.83 (0.49-1.40)	27	1.13 (0.60-2.12)	55	1.06 (0.70-1.62)
Q4	54	53	16,416	104	0.98 (0.70-1.38)	29	0.89 (0.51-1.58)	20	0.88 (0.42-1.85)	55	1.09 (0.70-1.70)
P for trend					0.61		0.70		0.86		0.29
Continuous, per 25 g/day incr.			65,980	415	1.04 (0.89-1.23)	131	1.03 (0.77-1.36)	88	1.12 (0.77-1.65)	193	1.02 (0.84-1.24)
Allium vegetables[f]											
Q1	6	4	17,832	120	1 (reference)	33	1 (reference)	31	1 (reference)	55	1 (reference)
Q2	19	20	14,814	80	0.90 (0.65-1.25)	26	1.08 (0.63-1.85)	15	0.68 (0.35-1.31)	39	0.92 (0.59-1.44)
Q3	31	33	16,778	100	0.98 (0.71-1.34)	33	1.20 (0.71-2.04)	17	0.62 (0.32-1.18)	49	1.03 (0.68-1.58)
Q4	55	55	16,556	115	1.07 (0.76-1.50)	39	1.39 (0.77-2.49)	25	0.79 (0.39-1.59)	50	1.02 (0.64-1.61)
P for trend					0.64		0.26		0.50		0.85
Continuous, per 25 g/day incr.			65,980	415	1.00 (0.86-1.16)	131	1.05 (0.83-1.34)	88	0.91 (0.65-1.27)	193	1.00 (0.81-1.22)

(continued)	Median (g/day)		Subcohort	HNC overall		OCC		OHPC		LC	
	Men	*Women*	Person-time at risk (yrs)	No. cases	RR (95% CI)	No. cases	RR (95% CI)	No. cases	RR (95% CI)	No. cases	RR (95% CI)
Legumes[f]											
Q1	13	11	16,434	109	1 (reference)	37	1 (reference)	21	1 (reference)	50	1 (reference)
Q2	24	21	16,715	88	0.81 (0.58-1.13)	27	0.75 (0.44-1.27)	21	0.98 (0.50-1.92)	39	0.78 (0.50-1.22)
Q3	36	32	16,558	109	1.01 (0.75-1.38)	25	0.74 (0.44-1.25)	24	1.18 (0.63-2.19)	60	1.16 (0.77-1.74)
Q4	58	52	16,274	109	1.07 (0.76-1.49)	42	1.33 (0.79-2.24)	22	1.10 (0.54-2.20)	44	0.88 (0.56-1.39)
P for trend					0.41		0.20		0.70		0.92
Continuous, per 25 g/day incr.			65,980	415	1.03 (0.88-1.21)	131	1.17 (0.90-1.51)	88	0.93 (0.69-1.26)	193	0.98 (0.81-1.18)
Total fruits[g]											
Q1	41	75	16,231	168	1 (reference)	52	1 (reference)	39	1 (reference)	75	1 (reference)
Q2	109	144	16,525	90	0.64 (0.48-0.87)	28	0.63 (0.39-1.03)	21	0.68 (0.38-1.21)	40	0.63 (0.42-0.96)
Q3	166	212	16,654	71	0.62 (0.45-0.85)	30	0.79 (0.48-1.31)[j]	12	0.51 (0.26-1.01)	29	0.55 (0.35-0.88)
Q4	271	325	16,571	86	0.77 (0.56-1.04)	21	0.56 (0.33-0.97)	16	0.73 (0.39-1.37)	49	0.95 (0.63-1.42)
P for trend					0.09		0.07		0.25		0.80
Continuous, per 25 g/day incr.			65,980	415	0.97 (0.94-1.00)[h]	131	0.95 (0.91-1.01)	88	0.97 (0.89-1.05)	193	0.99 (0.95-1.03)
Citrus fruits[f]											
Q1	0	7	16,621	144	1 (reference)	45	1 (reference)	33	1 (reference)	64	1 (reference)
Q2	16	37	16,430	111	0.81 (0.60-1.09)	31	0.73 (0.44-1.21)	20	0.65 (0.35-1.22)	59	0.94 (0.64-1.39)
Q3	58	83	17,792	87	0.62 (0.45-0.85)	28	0.67 (0.39-1.15)	15	0.50 (0.25-1.01)	44	0.65 (0.42-0.99)
Q4	126	167	15,137	73	0.71 (0.49-1.03)[i]	27	0.94 (0.52-1.71)	20	1.08 (0.57-2.04)	26	0.48 (0.28-0.83)
P for trend					0.05		0.95		0.78		0.003
Continuous, per 25 g/day incr.			65,980	415	1.00 (0.94-1.06)	131	1.02 (0.92-1.13)	88	1.12 (0.99-1.25)	193	0.93 (0.85-1.02)

[a]Adjusted for age (years); sex; cigarette smoking (status (never/former/current), frequency (number of cigarettes per day; continuous, centered), duration (number of years; continuous, centered)); and alcohol consumption (grams ethanol per day; continuous).
[b]OCC: oral cavity cancer; OHPC: oro-/hypopharyngeal cancer; LC: laryngeal cancer.
[c]Abbreviations: RR: incidence rate ratio; CI: confidence interval; Q: quartile; incr.: increment.
[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 (grams per day; continuous).
[f]Additionally adjusted for total vegetable and fruit intake (grams per day; continuous).
[g]Additionally adjusted for total vegetable intake (grams per day; continuous).
[h]$P \geq 0.05$.
[i]The proportional hazards assumption was possibly violated for the exposure variable in this analysis; there was no statistically significant interaction between the exposure variable and time.
[j]The proportional hazards assumption was possibly violated for the exposure variable in this analysis; there was a statistically significant interaction between the exposure variable and time.

Table 3.4. Multivariable-adjusted[ab] associations between consumption of individual vegetable and fruit items and risk of head-neck cancer subtypes; Netherlands Cohort Study, 1986–2006

	Head-neck cancer cases			
	Overall (*N*=415)	**Subtypes**		
		OCC[c] (*N*=131)	**OHPC[c] (*N*=88)**	**LC[c] (*N*=193)**
	RR[de] (95% CI)	**RR (95% CI)**	**RR (95% CI)**	**RR (95% CI)**
Vegetable items, RRs per 25 g/day increment				
String/French beans	0.89 (0.70-1.14)[f]	1.12 (0.78-1.62)	0.80 (0.50-1.28)[f]	0.77 (0.55-1.07)
Cauliflower	0.99 (0.71-1.37)	0.97 (0.62-1.54)	1.18 (0.45-3.11)[f]	0.92 (0.64-1.30)
Lettuce	0.58 (0.35-0.95)	0.61 (0.28-1.34)	0.38 (0.11-1.39)	0.70 (0.36-1.33)
Carrots, cooked	0.84 (0.56-1.25)	0.72 (0.33-1.55)	1.42 (0.78-2.59)	0.71 (0.42-1.20)
Endive, cooked	1.02 (0.78-1.35)	1.42 (0.92-2.20)	0.89 (0.53-1.48)	0.86 (0.59-1.28)
Brussels sprouts	1.08 (0.71-1.64)	0.87 (0.39-1.96)	0.95 (0.41-2.22)	1.31 (0.79-2.17)
Sauerkraut	0.89 (0.53-1.51)	1.03 (0.42-2.51)	0.53 (0.17-1.58)	1.04 (0.55-1.99)
Tomatoes	1.04 (0.89-1.21)	0.98 (0.77-1.26)	1.24 (0.90-1.71)	0.97 (0.80-1.19)
Onion	0.93 (0.77-1.11)	0.93 (0.68-1.26)	0.91 (0.60-1.38)	0.92 (0.73-1.17)
Spinach	1.23 (0.90-1.69)	1.00 (0.58-1.72)	1.01 (0.54-1.90)	1.54 (1.03-2.29)
Beetroot	1.01 (0.69-1.48)	0.80 (0.40-1.61)	0.62 (0.21-1.90)	1.30 (0.90-1.87)
Kale	1.79 (0.83-3.84)	1.20 (0.28-5.13)	2.65 (0.62-11.29)	1.96 (0.78-4.91)
Fruit items, RRs per 25 g/day increment				
Apples and pears	1.03 (0.97-1.08)	1.00 (0.92-1.10)	0.90 (0.79-1.02)	1.07 (1.00-1.15)[g]
Strawberries	1.04 (0.70-1.53)	1.07 (0.61-1.90)	1.07 (0.48-2.35)	1.03 (0.57-1.84)
Oranges and fresh orange juice	0.99 (0.92-1.07)	1.01 (0.89-1.14)	1.16 (1.03-1.32)	0.90 (0.81-1.00)

[a]Adjusted for age (years); sex; cigarette smoking (status (never/former/current), frequency (number of cigarettes per day; continuous, centered), duration (number of years; continuous, centered)); alcohol consumption (grams ethanol per day; continuous); and total vegetable and fruit intake (grams per day; continuous).

[b]The total person-time at risk in the subcohort was 65,980 years.

[c]OCC: oral cavity cancer; OHPC: oro-/hypopharyngeal cancer; LC: laryngeal cancer.

[d]Abbreviations: RR: incidence rate ratio; CI: confidence interval.

[e]Continuous variables, RR per 25 g/day increment.

[f]The proportional hazards assumption was possibly violated for the exposure variable in this analysis; there was no statistically significant interaction between the exposure variable and time.

[g]$P<0.05$.

Total fruit consumption was also associated with decreased risks of HNC overall and of all subtypes. All point estimates were below unity, but no clear trend was observed and statistically significant associations were only found when comparing Q3 vs. Q1 and Q2 vs. Q1 in HNC overall and in LC, and Q4 vs. Q1 in OCC (Table 3.3). Citrus fruits were clearly statistically significantly associated with a decreased risk of LC (RR for Q4 vs. Q1: 0.48, 95% CI 0.28-0.83, *P* trend=0.003).

With respect to individual vegetable and fruit items (Table 3.4), only lettuce was—after adjustment for total vegetable and fruit consumption—statistically significantly associated with a decreased HNC risk, namely for HNC overall (RR per 25 grams/day: 0.58, 95% CI 0.35-0.95). After adjustment for total vegetable and fruit intake, we found positive associations for consumption of oranges and fresh orange juice and OHPC (RR per 25 grams/day: 1.16, 95% CI 1.03-1.32), spinach and LC (RR per 25 grams/day: 1.54, 95% CI 1.03-2.29), and apples and pears and LC (RR per 25 grams/day: 1.07, 95% CI 1.00-1.15).

Stratified analyses

Results of the multivariable-adjusted stratified analyses are presented in Table 3.5. Among current smokers, we found statistically significantly decreased risks of HNC overall for participants with the highest vegetable and fruit consumption (those in Q4 (RR: 0.52, 95% CI 0.31-0.88) and Q3 (RR: 0.54, 95% CI 0.34-0.86)) compared to current smokers in Q1 as a reference group. No statistically significant interaction was found between categories of cigarette smoking status and vegetable and fruit intake (Q1-Q4) for HNC overall (*P* interaction=0.10).

We found a similar pattern for alcohol consumption, with statistically significantly decreased risks of HNC overall among participants who drank ≥15 grams/day and had the highest vegetable and fruit consumption (those in Q4 (RR: 0.48, 95% CI 0.30-0.77) and Q3 (RR: 0.47, 95% CI 0.30-0.74)). No statistically significant interaction was found between alcohol consumption and vegetable and fruit intake (Q1-Q4) for HNC overall (*P* interaction=0.09).

Table 3.5. Stratified analyses of sex/cigarette smoking/alcohol consumption and quartiles of vegetable and fruit consumption and risk (multivariable-adjusted[a] associations) of head-neck cancer overall; Netherlands Cohort Study, 1986–2006

	Head-neck cancer overall			
	Vegetable and fruit consumption			
	Q1[b]	Q2	Q3	Q4
Sex				
M				
Cases/person-time at risk (years)	110/7,493	82/7,752	65/7,553	63/7,871
RR (95% CI)	1 (ref)	0.82 (0.58-1.15)	0.63 (0.43-0.90)	0.63 (0.43-0.93)
F				
Cases/person-time at risk (years)	40/8,786	17/8,786	21/8,902	17/8,837
RR (95% CI)	1 (ref)	0.48 (0.26-0.89)	0.59 (0.33-1.06)	0.48 (0.25-0.92)
P for interaction[c]	0.41			
Cigarette smoking				
Never				
Cases/person-time at risk (years)	14/5,948	13/6,196	17/6,596	12/7,095
RR (95% CI)	1 (ref)	0.84 (0.38-1.85)	1.20 (0.57-2.53)	0.54 (0.22-1.29)
Former				
Cases/person-time at risk (years)	39/4,919	22/6,179	27/6,037	37/6,588
RR (95% CI)	1 (ref)	0.45 (0.26-0.79)	0.59 (0.34-1.00)	0.72 (0.44-1.18)
Current				
Cases/person-time at risk (years)	97/5,413	64/4,163	42/3,822	31/3,026
RR (95% CI)	1 (ref)	0.89 (0.60-1.31)	0.54 (0.34-0.86)	0.52 (0.31-0.88)
P for interaction	0.10			
Alcohol consumption				
Abstainers				
Cases/person-time at risk (years)	14/4,228	12/3,784	5/3,676	10/3,720
RR (95% CI)	1 (ref)	1.06 (0.47-2.35)	0.47 (0.17-1.36)	0.89 (0.35-2.24)
>0-15 (g/day)				
Cases/person-time at risk (years)	50/8,340	34/8,819	41/8,553	34/8,700
RR (95% CI)	1 (ref)	0.69 (0.43-1.11)	0.97 (0.62-1.53)	0.79 (0.49-1.28)
≥15 (g/day)				
Cases/person-time at risk (years)	86/3,711	53/3,935	40/4,225	36/4,287
RR (95% CI)	1 (ref)	0.71 (0.46-1.09)	0.47 (0.30-0.74)	0.48 (0.30-0.77)
P for interaction	0.09			

[a]Mutually adjusted for age (years); sex; cigarette smoking (status (never/former/current), frequency (number of cigarettes per day; continuous, centered), duration (number of years; continuous, centered)); and alcohol consumption (grams ethanol per day; continuous).

[b]Abbreviations: RR: incidence rate ratio; CI: confidence interval; Q: quartile.

[c]*P* value for interaction based on cross-product terms in the Cox proportional hazards model and Wald test.

Discussion

In this large prospective cohort study, we found significant inverse associations between consumption of vegetables and fruits and risk of HNC. Total vegetable and fruit consumption was associated with a substantially decreased risk of 0.61 (95% CI 0.44-0.85; Q4 vs. Q1) for HNC overall; among subtypes, OCC was most strongly inversely associated with total vegetable and fruit intake. Total vegetable consumption was inversely associated with risk of HNC overall as well; associations with HNC subtypes did not reach statistical significance and were largely similar for OCC, OHPC, and LC. Total fruit consumption was associated with reduced risks—but not a consistent trend—of HNC overall and all subtypes; the strongest associations were with OCC. Associations between vegetable and fruit consumption and HNC risk were not clearly modified by cigarette smoking status or alcohol consumption.

Total vegetables and fruits, subgroups, and individual items

The results of our study are generally in line with those of previous studies (summarized in a systematic literature review by the World Cancer Research Fund[3]) which showed consistent inverse associations between intake of total vegetables and fruits and HNC risk.[5,7,9,10,27-29] However, the strength of these associations varied, possibly as a result of differences in study design, case definition and/or exposure assessment. In the NIH-AARP Diet and Health cohort, a hazard ratio (HR) of 0.71 (95% CI 0.55-0.92) for total fruit and vegetable intake was reported, comparing Q5 vs. Q1.[9] In the EPIC study, an estimated relative risk of 0.60 (95% CI 0.37-0.99; Q5 vs. Q1) was observed for consumption of total fruits and vegetables and risk of UADTC, which also includes esophageal cancer.[7] Furthermore, in the NIH-AARP cohort it was found that HNC risk might be more strongly inversely associated with vegetables than with fruits, which appears to be the same in our study. However, the EPIC study found that fruits had a stronger effect.

Regarding the difference we found among HNC subtypes, consumption of total fruit and vegetables, and of vegetables alone, was also most strongly associated with OCC in the NIH-AARP cohort[9]; other prospective studies did not examine OCC, OHPC, and LC separately. A meta-analysis of case-control studies showed that consumption of fruit and vegetables had stronger protective effects for mouth and pharynx cancer than for LC.[28]

Prospective data regarding vegetable and fruit subgroups and individual items and HNC risk shows inconsistent associations and differences from our results as well.[3,7-11] This may be because other studies handled different subgroup classifications, studied

other food items or adjusted for confounders in their analyses differently. We found that raw leafy vegetables and citrus fruits, when adjusted for total vegetable and fruit intake, may exert an additional effect on the risk of HNC overall and LC, respectively. The NIH-AARP cohort analysis[9] used botanical subgroup classifications and found significantly lower risks for HNC overall for leguminosae (beans, peas), rosaceae (apples, peaches, nectarines, strawberries), solanacea (peppers/tomatoes) and umbelliferae (carrots), but not for chenopodiaceae (raw spinach and cooked spinach), compositae (lettuce) and rutaceae (citrus fruit). In the prospective EPIC study[7], vegetable subgroups were not related to risk of UADTC (with the exception of intake of root vegetables in men). Raw (leafy) vegetables were not examined as a separate vegetable subgroup and citrus fruits were not statistically significantly associated with decreased UADTC-risk.[7] A prospective study of Norwegian men[11] found that oranges were significantly inversely related to upper aerogastric tract cancer, but the study only included 71 cases (28 oral and pharyngeal, 21 laryngeal, and 22 esophageal cancer cases). It found no effect for highest vs. lowest intake of lettuce. In conclusion, certain vegetable and fruit subgroups and items may offer stronger HNC risk reduction than others, but the evidence from prospective data remains inconclusive. Evidence from case-control studies regarding vegetable and fruit subgroups shows that raw vegetables and citrus fruits are consistently associated with reduced HNC risk estimates.[3]

Possible interaction between alcohol, smoking, and total vegetable and fruit intake

We found no significant differences in associations for vegetable and fruit intake and HNC overall among strata of cigarette smoking and alcohol consumption. Similar to our results, the NIH-AARP Diet and Health cohort and the EPIC study found no statistically significant differences for total vegetable and fruit intake and HNC risk by smoking status or alcohol consumption.[7,9] However, our study found that both current smokers and participants who drink ≥15 grams of alcohol per day while eating the highest amount of vegetables and fruits had the lowest risk of HNC overall. Our non-significant findings regarding the interaction might be due to limited power to detect a significant deviation from the multiplicative model.

Mechanisms of action

Several mechanisms of action that vegetables and fruits have on HNC risk have been described, mainly the potential anticarcinogenic properties of numerous components present in vegetables and fruits.[3,5] These components include vitamins, fiber, folate, flavonoids and carotenoids, some of which are potential antioxidants and might play a

role in several processes, such as protection against oxidative stress and DNA repair. These substances may have an indirect, systemic effect (i.e., they become available in the body after being metabolized and subsequently act in numerous processes across the body), but they may also exert their effect on HNC carcinogenesis through direct exposure to tissue in the head-neck area. This is a possible explanation for the differential risk among HNC subtypes, as the larynx is being minimally exposed to foods and drinks compared with the oral cavity and pharynx.

There might be other—less described, yet potentially interesting—mechanisms for the effect of vegetables and fruits on HNC risk. One potential mechanism regarding the association between vegetables, fruits and HNC risk might be the beneficial effect that vegetables—especially raw vegetables—and fruits have on the body's acid-base balance by promoting alkalinity, thereby possibly influencing tumor growth.[30,31] Furthermore, a diet high in vegetables and fruits may influence the process of low-grade, chronic inflammation in the body, which might be involved in cancer risk as well.[32-34] Given that perspective, it might also be interesting to examine dietary patterns and HNC risk, since people who eat more vegetables and fruits might have a generally healthier lifestyle, reflecting another dietary pattern than that of people who consume more animal and/or processed foods.[35,36] So far, a dietary pattern reflecting relatively high fruit/vegetable intake and low intake of animal/processed foods has been associated with reduced HNC risk.[35-37]

Strengths and limitations

Important strengths of our study are the prospective nature and completeness and duration of follow-up. Given our large number of cases, we were able to examine the effect of vegetable and fruit consumption not only on overall HNC risk but also on the risk of HNC subtypes. Furthermore, we were able to thoroughly adjust for confounding by the main HNC risk factors (smoking and alcohol consumption), including information on smoking duration.

A possible limitation of our study is the single measurement of exposure data by our questionnaire. Although vegetable and fruit intake was extensively addressed, the FFQ may have provided only moderately accurate estimates of vegetable and fruit consumption. In addition, we believe the FFQ was given to people in an age group that had stable dietary habits at baseline, but it is nevertheless possible that participants changed their dietary habits since 1986. As a consequence, bias due to random misclassification may have occurred, possibly resulting in an underestimation of the effect of vegetables and fruits on HNC risk. The true risk lowering effect of vegetable and fruit consumption on HNC may therefore be even stronger than we found.

Furthermore, although we have detailed information about alcohol consumption and cigarette smoking in our cohort and were therefore able to adjust for these confounders extensively, we cannot exclude the presence of some residual confounding by smoking and/or alcohol.[27] Age- and sex-adjusted results were, however, largely comparable with multivariable-adjusted results. Residual confounding may also have occurred due to other potential confounders, but as we investigated many of these, we presume this to be limited as well. Finally, for reasons of efficiency, we used listwise deletion to handle missing data on confounders. This statistical approach resulted in only a minor loss of power when compared to the use of multiple imputation, but might have led to bias as a result of removing data with missing values.[38]

Our study lacks information on HPV infection, which has been associated with HNC risk.[4,39-41] Bias due to potential misclassification with regard to HPV is therefore possible, which makes the lack of information on HPV a limitation of our study. Other factors we did not take into account are the use of drugs and oral hygiene.

Finally, though we wanted to examine the role of vegetable and fruit consumption in HNC subtypes, we did not investigate HNC located in the major salivary glands, nasal cavity, paranasal sinuses and nasopharynx because of a presumably different etiology as well as low case numbers.

Conclusions

The present study, the second largest prospective study regarding this topic so far, confirms and further establishes that vegetable and fruit consumption is associated with reduced risk of developing HNC and HNC subtypes. Specific groups of vegetables and fruits may have an additional HNC risk reducing effect. In future research, it might be interesting to further elucidate certain mechanisms which may or may not be involved in (head-neck) cancer risk and development.

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Supplemental Table 3.1. Daily median intake and interquartile ranges of specific items of vegetables and fruits of cases and subcohort members; Netherlands Cohort Study, 1986–2006

	Subcohort (*N*=3,898)[a]			HNC[b] overall (*N*=415)[a]		
	Nonusers (%)	Users: daily intake (g)		Nonusers (%)	Users: daily intake (g)	
Food group or food item[c]		**Median**	**P25-P75**		**Median**	**P25-P75**
Total vegetables	0	179	137-229	0	175	126-223
String/French beans	2	17	10-25	3	17	10-25
Cauliflower	7	13	8-20	12	14	9-20
Lettuce	9	7	4-11	14	7	4-9
Carrots, cooked	12	8	4-13	17	8	4-12
Endive, cooked	14	12	7-18	14	10	6-20
Brussels sprouts	15	8	5-12	15	8	5-13
Sauerkraut	16	6	3-9	14	5	3-9
Tomatoes	18	24	14-33	21	24	14-35
Onion	20	22	11-33	22	22	11-33
Spinach	20	10	6-16	17	11	6-17
Beetroot	25	9	5-14	30	8	5-13
Kale	25	4	2-5	24	4	2-6
Cabbage	29	8	4-13	30	8	4-13
Leek	32	10	5-17	32	11	6-17
Dried pulses[d]	39	11	4-17	38	11	6-19
Mushrooms	39	4	4-9	40	4	4-9
Broad beans	43	6	3-10	35	6	4-11
Sweet peppers	47	4	3-6	52	4	3-5
Endive, raw	56	4	2-7	59	4	2-6
Rhubarb	59	3	1-6	65	3	1-6
Carrots, raw	68	5	2-10	77	4	1-9
Gherkins	70	3	1-6	67	2	1-5
Vegetable juices[d]	90	12	5-23	90	12	5-23
Total fruits	1	156	95-234	3	113	56-203
Apples and pears	13	80	45-116	21	45	18-116
Strawberries	14	7	4-11	16	5	2-9
Oranges and fresh orange juice	16	39	15-83	27	26	9-83
Grapes	37	3	1-7	44	3	1-9
Mandarins	41	4	2-8	49	3	2-8
Bananas	47	11	4-19	54	11	4-19
Processed fruit juices[d]	50	23	12-61	62	23	12-60
Grapefruits and fresh grapefruit juices	71	16	5-40	77	16	4-40
Raisins and other dried fruits	75	1	1-3	86	1	1-2

[a]The number of subcohort members and head-neck cancer cases used in age- and sex-adjusted and multivariable-adjusted analyses of vegetable and fruit consumption.

[b]HNC: head-neck cancer.

[c]Values are given as medians and interquartile ranges because of the right-skewed distribution of vegetable and fruit items.

[d]Dried pulses and vegetable juices are not included in total vegetable consumption. Processed fruit juices are not included in total fruit consumption.

Chapter 4

Vitamin and carotenoid intake and the risk of head-neck cancer subtypes in the Netherlands Cohort Study

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Abstract

Background

Head-neck cancer (HNC) is the seventh most common type of cancer worldwide. Evidence regarding the potential protective effect of vitamins and carotenoids on HNC is limited and mostly based on case-control studies. We evaluated the association of the intake of dietary vitamins C and E (including supplementation), and the most common carotenoids (alpha-carotene, beta-carotene, lutein/zeaxanthin, lycopene, and beta-cryptoxanthin) on the risk of HNC (subtypes) within the Netherlands Cohort Study.

Methods

At baseline in 1986, 120,852 participants completed a food frequency questionnaire. For efficiency reasons, a case-cohort design was used. A subcohort was randomly selected from the total cohort. After 20.3 years of follow-up, 3,898 subcohort members and 415 HNC cases (131 oral cavity cancer (OCC), 88 oro-/hypopharyngeal cancer (OHPC), and 193 laryngeal cancer (LC)) were available for analysis. Incidence rate ratios (RRs) and 95% confidence intervals (CI) for highest (Q4) vs. lowest quartile (Q1) of vitamin and carotenoid intake were estimated using the Cox proportional hazards model.

Results

A strong inverse association was found between vitamin C and HNC overall (multivariable-adjusted RR for Q4 vs. Q1: 0.39, 95% CI 0.23-0.66, *P* trend<0.001), OCC (0.35, 95% CI 0.16-0.77, *P* trend<0.05) and OHPC (0.29, 95% CI 0.12-0.67, *P* trend<0.01). No statistically significant results were found for vitamin E, alpha-carotene, beta-carotene, lycopene, and lutein/zeaxanthin. The association of vitamin E and HNC was modified by alcohol status (*P* interaction=0.003), with lower risks in alcohol abstainers.

Conclusions

This study shows an inverse association between intake of vitamin C and the incidence of HNC (subtypes). Future research is recommended to investigate the underlying mechanisms and to confirm our results, which may be promising for the prevention of HNC.

Introduction

Head and neck cancer (HNC) is the seventh most commonly occurring type of cancer worldwide and the seventh most common cause of death from all cancers.[1,2] The estimated incidence worldwide is 600,000 people every year, with the highest incidence in males aged 60-74 year.[1,3]

Alcohol consumption, tobacco use and human papillomavirus infection (HPV) are identified as important risk factors for HNC.[2,4-9] An inverse association is implicated for fruit and vegetable intake and HNC risk, probably because they contain vitamins and carotenoids that exhibit antioxidant properties.[2,10-12] Antioxidants can protect the human body against free radicals that are associated with a higher cancer risk.[13-15] Besides, vitamins and carotenoids increase the apoptosis of cancer cells, decrease cell proliferation and maintain the normal DNA repair system. In addition, the nutrients are essential for normal cell differentiation.[16]

One prospective cohort study investigated the association between vitamins and carotenoids and the risk on HNC.[17] Despite the small number of cases among the 34,691 postmenopausal women, the results suggest that a high intake of antioxidants may be protective against HNC. A meta-analysis from 2013 provided evidence about the protective effect of carotenoids on HNC risk.[18] However, this study was mainly based on case-control studies. In addition, a pooled analysis from the INHANCE consortium, based on 12 case-control studies, provided evidence about the protective effect of vitamin C supplement use.[19] Finally, randomized controlled trials have shown inconsistent results between the association of vitamin E and beta-carotene supplementation and risk on HNC (subtypes).[20,21] Thus, the results of epidemiological data are inconclusive and are mainly based on case-control studies.

Therefore, we studied the association of dietary intake of vitamins C and E, the most common carotenoids (alpha-carotene, beta-carotene, lutein/zeaxanthin, lycopene, and beta-cryptoxanthin) and vitamin C and E supplements and the risk on HNC and HNC subtypes in a large prospective cohort study. The following hypotheses were investigated: 1) HNC risk is higher in participants with a low intake of vitamins and carotenoids; and 2) these risks are different for HNC subtypes, i.e., oral cavity cancer (OCC), oro-/hypopharyngeal cancer (OHPC), and laryngeal cancer (LC). Furthermore, we investigated the possible effect modification by smoking and alcohol consumption.

Methods

Study design and population

The study characteristics have been described in detail previously.[22] The Netherlands Cohort Study (NLCS) started in September 1986 and included 120,852 participants. The participants were aged 55-69 years at baseline and were derived from 204 Dutch municipal computerized population registries. Participants completed a questionnaire at baseline.

For efficiency reasons a case-cohort design was used. A total of 5,000 participants (2,411 men and 2,589 women) were randomly sampled from the total cohort at baseline to create a subcohort from which person-years at risk were estimated. Incident cases were derived from the entire cohort. All incident cases were monitored during the follow-up period of 20.3 years, through annual linkage to the Netherlands Cancer Registry and the Netherlands Pathology Registry. The completeness of this cancer follow-up is estimated to be ≥96%.[23]

Prevalent cancer cases at baseline were excluded from the analysis, except for skin cancer. Participants with incomplete and inconsistent dietary data and missing data on exposure or confounding variables were also excluded. Incomplete and inconsistent dietary data included 1) participants who left 60 or more out of 150 items blank and ate fewer than 35 items at least once per month and 2) participants who left one or more item blocks blank.[24] In total, 415 pathologically confirmed incident cases and 3,898 subcohort members were available for this study (Figure 4.1). As proposed by Hashibe et al.[25], 131 cases were classified as OCC, 88 as OHPC, and 193 cases were classified as LC. Furthermore, 3 cases were classified as oral cavity, pharynx unspecified, or overlapping.

Approval for the NLCS was obtained by the review boards of TNO Nutrition and Food Research Institute and Maastricht University. All participants were informed before participation.

Questionnaire data

At baseline, all participants completed a self-administered questionnaire, which purpose was to assess habitual food consumption, lifestyle, and other cancer risk factors preceding the start of the study.

The 150-item food frequency questionnaire (FFQ) was part of the baseline questionnaire. To make sure misclassification was minimized, an elaborate dietary assessment covering seasonal variations was necessary. The FFQ contained most fruits and vegetables (the main contributors of vitamins and carotenoids), which were eaten

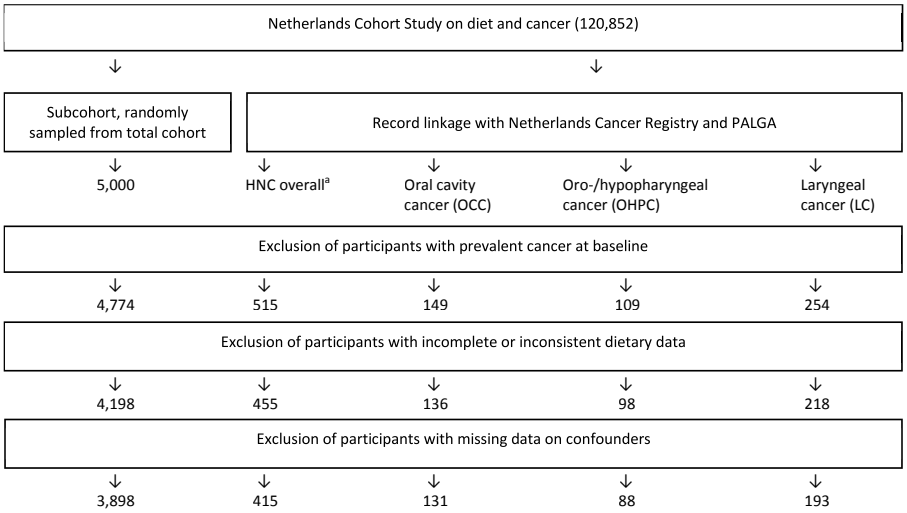

Figure 4.1. Flow diagram of the number of subcohort members and cases on whom the analyses were based. Abbreviation PALGA: nationwide network and registry of histopathology and cytopathology in the Netherlands. [a]Oral cavity cancer; oro-/hypopharyngeal cancer; oral cavity, pharynx unspecified or overlapping cancer; laryngeal cancer.

regularly in 1986, except for red cabbage, cucumber and chicory. Since broccoli was a rarely available vegetable in 1986, it was also excluded from the FFQ. The frequency of vegetable intake was divided into categories ranging from 'never or less than once per month' to '3-7 times per week'. However, a distinction was made between the intake of vegetables in the summer and in the winter. In addition, the consumption frequency of fruit ranged from 'never or less than once per month' to '6-7 times per week'. In addition, participants could fill out the number of fruits they ate per consumption. For cooked endive and string beans, participants were asked about their usual serving sizes (tablespoons and grams per person). The mean of those two vegetables was used as a representative of standard serving sizes of all leafy and solid vegetables respectively because a pilot study showed a correlation between serving sizes of different types of cooked vegetables. The serving size was multiplied with a vegetable-specific factor, to determine the mean daily intake in grams for vegetable consumption. The FFQ also included the self-reported use of supplements (vitamin supplements, drops, or others) during the five years prior to completion of the questionnaire. The type of supplement, brand, years of intake, and dose per day were asked. To reduce observer bias in coding or interpretation, data of the questionnaire was key-entered for incident cases and subcohort members, blinded with respect to case/subcohort status.

To calculate the daily intake of the vitamins C and E the Dutch food composition table was used.[26] The mean daily intake of alpha-carotene, beta-carotene, lutein + zeaxanthin, lycopene, and beta-cryptoxanthin was calculated with an alternative food

composition table, which was completed with data from manufacturers and recent literature.[11] Since most of these literature sources did not provide separate values for each of the carotenoids, lutein and zeaxanthin were taken together.

The FFQ was validated and tested for reproducibility.[24,27] Pearson correlation coefficients between the FFQ and the nine-day diet record were moderate; 0.58 for vitamin C and 0.52 for vitamin A (retinol + beta-carotene). Data was not available for the carotenoids, because there was no database for carotenoids separately at that time. However, the correlation of fruit, which is the main contributor of beta-cryptoxanthin, was 0.6 and for most macronutrients the correlation was between 0.65 and 0.80. The reproducibility study considered only a number of nutrients relevant at that time for cancer studies, e.g., vitamin A (retinol and beta-carotene) and vitamin C. The five-year reproducibility of vitamin A and C ranged from a Pearson correlation coefficient of 0.51 to 0.64, and can be considered as moderate.

Furthermore, detailed questions were asked about alcohol consumption and tobacco use at different moments during several years preceding the start of the NLCS.[28] Briefly, we asked for the alcohol consumption the year prior to completion of the questionnaire. In addition, we asked for alcohol consumption five years before baseline. The mean daily intake of ethanol (g/day) was calculated by using the Dutch food composition table. The questionnaire asked about smoking status, number of cigarettes smoked daily, and number of smoking years.

Statistical analysis

The Cox proportional hazards model was used to estimate age- and sex-adjusted incidence rate ratios (RR), multivariable-adjusted RRs and 95% confidence intervals (95% CI). Analyses were performed for the vitamins C and E, the carotenoids alpha-carotene, beta-carotene, lutein + zeaxanthin, lycopene, and beta-cryptoxanthin, and the use of any supplement containing vitamin C or E.

Participants were categorized, depending on the sex-specific distribution in the subcohort, according to quartiles (Q) of intake of these nutrients (Q1 for the lowest and Q4 for the highest quartile). Furthermore, participants were categorized as users or non-users of supplements containing vitamin C or E. Continuous variables were constructed for the vitamins and carotenoids, based on the difference between percentiles 75 and 25 in the subcohort. The increment ranged from 50 mg/day for vitamin C to 0.2 mg/day for beta-cryptoxanthin.

Vitamin and carotenoid intake of HNC cases diagnosed within the first two years of follow-up (*N*=33) were compared with the intake of HNC cases diagnosed later in follow-up (*N*=382). Early symptoms of HNC could influence the intake of vitamins and

carotenoids and therefore lead to reversed causation. Independent samples t-tests were performed to test for significant differences, because the normality assumption was still violated after transformation of the nutrient intakes. No differences were found between the intake of vitamins and carotenoids between the early cases and the cases diagnosed later in follow-up, with a *P* value ranging from 0.98 for lycopene (SD: 0.16, median value of 0.83 vs. 0.75 mg/day respectively) to 0.23 for alpha-carotene (SD: 0.09, median value of 0.43 vs. 0.52 mg/day, respectively). In addition, sensitivity analyses were performed in which the first two years of follow-up were excluded. No differences were found between the results for the period with exclusion of the first two years of follow-up and the total follow-up period (Supplemental Table 4.1). Therefore, the total follow-up period was included in the analyses.

Person-years at risk were calculated from baseline until time of diagnosis of HNC, loss to follow-up, end of follow-up, emigration, or death, whichever occurred first. Predefined confounders were sex; age (years); alcohol consumption (grams/day); cigarette smoking status (never/former/current), frequency (*N*/day), and duration (years). The variables smoking frequency and duration were centered, as proposed by Leffondré et al.[29] The categorical variable family history of HNC and the continuous variables BMI; level of education; intake of fish, red meat, and meat products (all grams/day); non-occupational physical activity; and total daily energy intake were considered as potential confounders.[2,30] Confounders had to change the RR of vitamin C or E (continuous variable) with HNC overall or any of the HNC subtypes with at least 10 percent, by using a backwards stepwise procedure. This resulted in a final model which contained the predefined confounders and total daily energy intake (kcal/day).

The proportional hazard (PH) assumption was tested using the scaled Schoenfeld residuals for every covariate.[31] When the PH assumption was violated, a time-varying covariate was introduced into the model and tested for significance. Current smoking did not meet the PH assumption for all HNC overall analyses, with a significant time-varying covariate. It is plausible that the interaction with time can be explained by participants who stopped smoking during the follow-up period. Therefore, the time-varying covariate for current smoking was also added in the model for HNC subtypes. A more elaborate explanation about this time-varying covariate is described elsewhere.[28]

To determine the adjusted RR of one nutrient, the independent variable was mutually adjusted for the other nutrients. In addition, *P* trend was calculated by using the quartiles as continuous terms for the independent variables in the Cox proportional hazards model. The Wald test was used to evaluate this trend.

To detect possible interaction by sex, smoking and alcohol consumption, a test for interaction was performed, using the interaction term in the Cox proportional hazards

model and Wald test for HNC overall cases. Furthermore, stratified analyses were performed for smoking status (never/former/current) and alcohol consumption (0 g/day; >0-15 g/day; >15 g/day).

All *P* values were based on two-sided tests and were considered statistically significant if *P*<0.05. Analyses were performed using the STATA software package (STATA, version 11.1; Stata Corporation, College Station, Texas, USA).

Results

Baseline characteristics

Cases were more frequently men (77.1%) than women (Table 4.1). Among subcohort members, almost half (49.3%) of the participants were men. Compared with cases, the daily median intake of most vitamins and carotenoids was higher among subcohort members, e.g., the intake of vitamin C of subcohort members was 96.1 mg/day vs. 82.3 mg/day for HNC overall. There were more users of vitamin E supplements among subcohort members (6.5%) than HNC overall cases (5.5%). However, the use of vitamin C supplements was similar among subcohort members and HNC overall cases (10.6%).

HNC overall cases were more often current smokers (56.4%) with a higher number of pack-years (34.1) than subcohort members (26.8% current smokers and 22.7 pack-years). Furthermore, with respect to alcohol use, subcohort members were more often abstainers and had a substantially lower daily ethanol intake than cases.

Main analyses

Age- and sex-adjusted analyses (Table 4.2) showed strong inverse associations, which were mostly less strong after multivariable-adjusted analyses (Table 4.3).

Vitamin C intake was associated with a significant age- and sex-adjusted decreased risk of HNC overall (RR for Q4 vs. Q1: 0.45, 95% CI 0.34-0.61, *P* trend<0.001) and of the subtypes OCC (RR for Q4 vs. Q1: 0.43, 95% CI 0.25-0.73, *P* trend=0.005), OHPC (RR for Q4 vs. Q1: 0.50, 95% CI 0.29-0.87, *P* trend=0.02), and LC (RR for Q4 vs. Q1: 0.46, 95% CI 0.29-0.96, *P* trend<0.001). In multivariable-adjusted analyses, vitamin C intake was still significantly associated with a decreased risk of HNC overall (RR for Q4 vs. Q1: 0.39, 95% CI 0.23-0.66, *P* trend<0.001), OCC (RR for Q4 vs. Q1: 0.35, 95% CI 0.16-0.77, *P* trend=0.03) and OHPC (RR for Q4 vs. Q1: 0.29, 95% CI 0.12-0.67, *P* trend=0.003). However, the association between vitamin C and LC was not statically significant in the multivariable-adjusted analyses.

In age- and sex-adjusted analyses, the *P* trend was only statistically significant for the association of beta-cryptoxanthin intake and HNC overall (*P* trend<0.001) and LC (*P* trend=0.002). No statistically significant associations were observed between intake of vitamin E, alpha-carotene, beta-carotene, lutein + zeaxanthin, lycopene and overall risk of HNC and the subtypes OCC, OHPC, and LC in age- and sex-adjusted analyses as well as multivariable analyses. However, most associations were below unity. Furthermore, after multivariable-adjusted analyses, the *P* trend was not statistically significant for the association between beta-cryptoxanthin intake and HNC overall and the subtypes.

Regarding the use of supplements containing vitamin C, the point estimations were mostly above 1 for users vs. non-users for HNC overall (RR: 1.18, 95% CI 0.76-1.85), OCC (RR: 0.99, 95% CI 0.53-1.86), OHPC (RR: 1.53, 95% CI 0.66-3.59), and LC (RR: 1.21, 95% CI 0.65-2.24), but none of those were significant. The intake of supplements containing vitamin E was inversely, however not significant, associated with HNC overall (RR: 0.89, 95% CI 0.51-1.54). In addition, there were no clear associations between vitamin E supplements and OCC (RR: 0.90, 95% CI 0.40-2.03), OHPC (RR: 0.87, 95% CI 0.28-2.72), and LC (RR: 1.07, 95% CI 0.47-2.43). However, the number of participants using supplements was small, e.g., *N*=44 for vitamin C and *N*=23 for vitamin E for HNC overall cases.

Stratified analyses

No statistically significant interaction was found between sex and the independent variables (*P* interaction ranging from 0.15 for alpha-carotene to 0.75 for lycopene) (Tables 4.4 and 4.5). Hence, analyses were performed for both sexes combined.

Statistically significant interactions were found between categories of alcohol consumption and vitamin E intake (*P* interaction=0.003) (Table 4.5). Alcohol abstainers showed decreased risks and the group of participants who drank ≥15 grams/day showed mostly increased risks compared with the reference group of participants who drank >0-15 grams/day. In alcohol abstainers a statistically significant inverse association between vitamin E intake and HNC risk were found in Q3 (RR: 0.29, 95% CI 0.10-0.85) and Q4 (RR: 0.15, 95% CI 0.03-0.64). In participants who drank >15 grams of alcohol per day, statistically significantly associations between vitamin E intake and HNC risk were found in Q1 (RR: 1.97, 95% CI 1.26-3.09) and Q2 (RR: 1.77, 95% CI 1.09-2.88). Furthermore, when stratified on smoking status, most results were below unity for never smokers and showed increased risks for current smokers, compared with never smokers in Q1.

Table 4.1. Characteristics of cases and subcohort members; Netherlands Cohort Study, 1986–2006

	Subcohort	Head-neck cancer cases			
		Overall	Subtypes		
			OCC[a]	OHPC[a]	LC[a]
Exposure variables and potential confounders[b]	**(*N*=3,898)[c]**	**(*N*=415)[c]**	**(*N*=131)[c]**	**(*N*=88)[c]**	**(*N*=193)[c]**
Age at baseline (years)	61.3 (4.2)	61.7 (4.1)	62.0 (4.2)	61.6 (4.0)	61.5 (4.0)
Sex: men (%)	49.3	77.1	57.3	73.9	92.8
Vitamin C[d] (mg/day)	96.1 (72.4-126.1)	82.3 (62.7-113.4)	86.1 (63.9-113.9)	75.5 (60.1-122.9)	82.8 (64.8-107.1)
Men	90.5 (68.5-121.2)	81.3 (62.5-110.0)	82.9 (31.7-111.6)	72.6 (59.4-118.9)	82.8 (64.6-107.1)
Women	100.8 (76.4-130.3)	87.0 (63.6-121.9)	95.3 (66.9-115.9)	80.5 (61.9-132.6)	83.5 (66.6-121.9)
Vitamin E[d] (mg/day)	12.2 (8.7-16.7)	11.7 (8.4-16.5)	10.9 (7.8-15.5)	12.8 (8.4-16.1)	12.7 (9.4-18.1)
Men	13.5 (9.8-18.3)	12.8 (9.5-17.8)	11.3 (8.3-17.1)	13.1 (9.6-16.4)	13.1 (9.5-18.2)
Women	11.0 (8.1-15.3)	9.3 (6.8-12.8)	9.8 (6.9-12.3)	9.3 (6.2-14.7)	8.0 (7.2-10.4)
Alpha-carotene[d] (mg/day)	0.56 (0.33-0.88)	0.51 (0.28-0.80)	0.45 (0.24-0.77)	0.53 (0.35-0.82)	0.53 (0.29-0.83)
Men	0.57 (0.34-0.89)	0.54 (0.29-0.84)	0.57 (0.24-0.93)	0.56 (0.36-0.86)	0.53 (0.29-0.83)
Women	0.55 (0.33-0.87)	0.42 (0.25-0.66)	0.40 (0.25-0.63)	0.49 (0.25-0.77)	0.49 (0.30-0.79)
Beta-carotene[d] (mg/day)	2.61 (1.92-3.49)	2.50 (1.81-3.29)	2.24 (1.65-3.38)	2.58 (1.82-3.24)	2.56 (1.99-3.29)
Men	2.66 (1.99-3.53)	2.58 (1.92-3.45)	2.57 (1.70-3.73)	2.58 (1.89-3.32)	2.58 (2.01-3.31)
Women	2.57 (1.86-3.45)	2.22 (1.54-3.03)	1.97 (1.58-2.89)	2.57 (1.68-3.10)	2.32 (1.54-3.05)
Lutein/zeaxanthin[d] (mg/day)	2.30 (1.74-2.92)	2.21 (1.68-2.92)	2.14 (1.61-2.88)	2.13 (1.75-2.83)	2.38 (1.76-3.06)
Men	2.35 (1.78-2.96)	2.26 (1.74-3.04)	2.15 (1.67-2.91)	2.09 (1.76-2.91)	2.43 (1.76-3.11)
Women	2.25 (1.69-2.89)	2.13 (1.52-2.69)	2.09 (1.52-2.78)	2.21 (1.63-2.75)	1.99 (1.58-2.46)
Beta-cryptoxanthin[d] (mg/day)	0.13 (0.05-0.26)	0.07 (0.02-0.20)	0.10 (0.02-0.22)	0.05 (0.02-0.25)	0.06 (0.02-0.18)
Men	0.10 (0.03-0.23)	0.06 (0.02-0.18)	0.05 (0.02-0.19)	0.05 (0.02-0.18)	0.06 (0.02-0.17)
Women	0.17 (0.07-0.28)	0.12 (0.04-0.29)	0.13 (0.04-0.31)	0.08 (0.01-0.37)	0.11 (0.04-0.29)
Lycopene[d] (mg/day)	0.80 (0.41-1.31)	0.76 (0.35-1.36)	0.83 (0.45-1.42)	0.72 (0.35-1.41)	0.73 (0.32-1.20)
Men	0.74 (0.35-1.21)	0.72 (0.33-1.29)	0.98 (0.47-1.46)	0.70 (0.35-1.34)	0.65 (0.28-1.20)
Women	0.88 (0.48-1.42)	0.92 (0.49-1.52)	0.72 (0.42-1.41)	1.04 (0.47-1.63)	1.08 (0.91-1.42)
Supplement use (%)					
Vitamin C	10.6	10.6	9.9	13.6	9.8
Vitamin E	6.5	5.5	5.3	6.8	5.2
Beta-carotene	0.05	-	-	-	-

(continued)	**Subcohort**	**Head-neck cancer cases**			
		Overall	**Subtypes**		
			OCC	**OHPC**	**LC**
Exposure variables and potential confounders	**(*N*=3,898)**	**(*N*=415)**	**(*N*=131)**	**(*N*=88)**	**(*N*=193)**
Cigarette smoking status (%)					
Never smoker	36.8	13.5	29.0	8.0	5.7
Former smoker	36.4	30.1	25.2	29.6	34.2
Current smoker	26.8	56.4	45.8	62.5	60.1
Ever cigarette smokers:					
Frequency of cigarette smoking (*N*/day)	15.3 (10.2)	19.5 (11.0)	20.4 (11.9)	21.1 (12.7)	18.4 (9.5)
Duration of cigarette smoking (years)	31.6 (12.2)	38.6 (9.9)	36.8 (10.1)	38.0 (10.3)	39.7 (9.6)
Pack-years of cigarette smoking (*N*)	22.7 (17.6)	34.1 (21.3)	34.9 (23.6)	36.2 (23.5)	32.8 (18.9)
Abstainer from alcohol (%)	23.6	9.9	8.4	12.5	9.8
Alcohol consumers: ethanol intake (g/day)	13.6 (15.1)	26.9 (26.2)	26.7 (27.3)	34.9 (31.4)	23.5 (22.1)
Level of education (%)					
Primary (men)	27.9 (23.4)	28.2 (27.7)	22.1 (17.3)	23.0 (21.5)	34.0 (34.5)
Lower vocational (men)	21.5 (20.0)	17.5 (16.4)	16.8 (13.3)	17.2 (16.9)	18.3 (17.5)
Secondary and medium vocational (men)	36.1 (36.8)	35.2 (34.9)	39.7 (42.7)	37.9 (33.9)	31.4 (32.2)
University and higher vocational (men)	14.6 (19.8)	19.2 (21.1)	21.4 (26.7)	21.8 (27.7)	16.2 (15.8)
Body mass index (kg/m^2)	25.0 (3.1)	24.8 (2.7)	25.0 (3.0)	24.4 (2.6)	25.0 (2.6)
Non occupational physical activity (min/day)	72.8 (60)	71.2 (58.3)	67.2 (59.5)	65.7 (55.2)	75.6 (58.1)
Total daily energy intake (kcal/day)					
Men	2,162 (498)	2,168 (500)	2,083 (557)	2,168 (431)	2,208 (494)
Women	1,687 (390)	1,628 (464)	1,587 (490)	1,710 (384)	1,671 (440)
Red meat consumption (g/day)	84.2 (61.7-107.4)	85.8 (65.7-111.1)	81.4 (58.0-106.1)	83.7 (51.6-111.3)	90.8 (70.8-117.2)
Family history of HNC (%)	2.0	1.7	1.5	1.1	1.6

[a]OCC: oral cavity cancer; OHPC: oro-/hypopharyngeal cancer; LC: laryngeal cancer.

[b]Values are given as mean (SD); for categorical variables, *N* (%) is presented. For dietary variables, medians and interquartile ranges (P25-P75) are shown because of their right-skewed distribution.

[c]The number of subcohort members or cases used in age- and sex-adjusted and multivariable-adjusted analyses of vitamin and carotenoid intake.

[d]Nutrient from food intake only.

Table 4.2. Age- and sex-adjusted associations between vitamin and carotenoid intake and risk of head-neck cancer subtypes; Netherlands Cohort Study, 1986–2006

			Subcohort	Head-neck cancer cases							
				Overall		Subtypes					
						OCC[a]		OHPC[a]		LC[a]	
	Median (mg/day)		Person-time	No.	RR[b] (95% CI)	No.	RR (95% CI)	No.	RR (95% CI)	No.	RR (95% CI)
	Men	*Women*	at risk (yrs)	cases		cases		cases		cases	
Vitamin C[c]											
Q1	55.2	63.5	16,281	150	1 (reference)	46	1 (reference)	39	1 (reference)	63	1 (reference)
Q2	79.8	89.2	16,490	101	0.63 (0.48-0.84)	28	0.58 (0.36-0.94)	18	0.44 (0.25-0.77)	54	0.80 (0.54-1.18)
Q3	103.3	113.8	16,661	91	0.57 (0.43-0.76)[e]	36	0.75 (0.48-1.17)[e]	10	0.24 (0.12-0.49)	45	0.66 (0.44-1.00)
Q4	144.8	153.3	16,548	73	0.45 (0.34-0.61)	21	0.43 (0.25-0.73)	21	0.50 (0.29-0.87)	31	0.46 (0.29-0.72)
P for trend[d]					<0.001		0.005		0.02		<0.001
Continuous, per 50 mg/day incr.			65,980	415	0.75 (0.64-0.88)	131	0.71 (0.55-0.90)	88	0.84 (0.57-1.23)	193	0.76 (0.60-0.96)
Vitamin E[c]											
Q1	7.6	6.5	16,054	127	1 (reference)	43	1 (reference)	25	1 (reference)	56	1 (reference)
Q2	11.5	9.4	16,588	111	0.86 (0.65-1.13)	38	0.88 (0.56-1.37)	23	0.90 (0.50-1.62)	50	0.87 (0.58-1.30)
Q3	15.7	12.9	16,537	91	0.70 (0.53-0.94)	29	0.67 (0.41-1.08)	20	0.79 (0.43-1.43)	42	0.74 (0.48-1.12)
Q4	22.2	18.4	16,801	86	0.65 (0.48-0.87)[e]	21	0.47 (0.28-0.80)	20	0.76 (0.42-1.39)	45	0.76 (0.51-1.15)[f]
P for trend					0.002		0.003		0.35		0.18
Continuous, per 8.0 mg/day incr.			65,980	415	0.79 (0.69-0.92)	131	0.70 (0.53-0.94)	88	0.84 (0.62-1.14)	193	0.85 (0.70-1.02)
Alpha-carotene[c]											
Q1	0.22	0.20	16,069	124	1 (reference)	43	1 (reference)	20	1 (reference)	58	1 (reference)
Q2	0.45	0.43	16,608	110	0.85 (0.64-1.12)	35	0.78 (0.50-1.24)	27	1.29 (0.71-2.31)	48	0.79 (0.53-1.17)
Q3	0.70	0.68	16,478	93	0.72 (0.54-0.96)	25	0.56 (0.34-0.93)	22	1.06 (0.57-1.96)	46	0.76 (0.51-1.14)
Q4	1.15	1.15	16,824	88	0.67 (0.50-0.90)	28	0.63 (0.38-1.02)	19	0.90 (0.48-1.71)	41	0.67 (0.44-1.01)
P for trend					0.008		0.06		0.51		0.08
Continuous, per 0.5 mg/day incr.			65,980	415	0.86 (0.75-0.98)	131	0.80 (0.64-1.00)	88	0.93 (0.73-1.19)	193	0.89 (0.73-1.08)
Beta-carotene[c]											
Q1	1.57	1.47	16,031	126	1 (reference)	46	1 (reference)	29	1 (reference)	48	1 (reference)
Q2	2.32	2.21	16,615	103	0.78 (0.59-1.04)	33	0.69 (0.44-1.10)	15	0.50 (0.26-0.93)	55	1.09 (0.73-1.64)
Q3	3.06	2.97	16,554	99	0.76 (0.57-1.01)	21	0.44 (0.26-0.75)	27	0.90 (0.53-1.53)	51	1.02 (0.68-1.54)
Q4	4.31	4.27	16,780	87	0.66 (0.49-0.88)	31	0.65 (0.41-1.03)	17	0.56 (0.30-1.02)	39	0.77 (0.49-1.19)
P for trend					0.008		0.07		0.19		0.16

(continued)	Median (mg/day)		Person-time at risk (yrs)	No. cases	RR (95% CI)	No. cases	RR (95% CI)	No. cases	RR (95% CI)	No. cases	RR (95% CI)
Continuous, per 1.5 mg/day incr.			65,980	415	0.85 (0.74-0.98)	131	0.79 (0.06-1.00)	88	0.87 (0.65-1.17)	193	0.91 (0.75-1.10)
Lutein/zeaxanthin[c]											
Q1	1.50	1.38	16,095	124	1 (reference)	44	1 (reference)	24	1 (reference)	54	1 (reference)
Q2	2.05	1.94	16,681	100	0.78 (0.59-1.03)	31	0.68 (0.43-1.09)	29	1.17 (0.67-2.02)	39	0.70 (0.45-1.07)
Q3	2.62	2.52	16,539	89	0.71 (0.53-0.95)	26	0.59 (0.36-0.96)	15	0.61 (0.32-1.18)	48	0.87 (0.58-1.31)
Q4	3.55	3.49	16,665	102	0.80 (0.60-1.06)	30	0.67 (0.42-1.08)	20	0.81 (0.44-1.48)	52	0.93 (0.62-1.39)
P for trend					0.16		0.13		0.24		0.94
Continuous, per 1.0 mg/day incr.			65,980	415	0.94 (0.84-1.05)	131	0.91 (0.74-1.11)	88	0.85 (0.67-1.09)	193	1.01 (0.87-1.16)
Lycopene[c]											
Q1	0.16	0.22	16,141	111	1 (reference)	30	1 (reference)	23	1 (reference)	57	1 (reference)
Q2	0.53	0.69	16,678	97	0.84 (0.62-1.12)	35	1.12 (0.68-1.84)	22	0.92 (0.51-1.66)	40	0.67 (0.44-1.02)
Q3	0.95	1.12	16,687	91	0.78 (0.58-1.05)	26	0.82 (0.48-1.40)	16	0.66 (0.35-1.27)	48	0.81 (0.54-1.21)
Q4	1.70	2.07	16,474	116	1.01 (0.76-1.34)	40	1.30 (0.81-2.11)	27	1.14 (0.65-2.00)	48	0.81 (0.54-1.21)
P for trend					0.73		0.35		0.64		0.57
Continuous, per 0.9 mg/day incr.			65,980	415	1.01 (0.96-1.06)	131	1.05 (0.99-1.11)	88	1.01 (0.93-1.11)	193	0.96 (0.87-1.06)
Beta-cryptoxanthin[c]											
Q1	0.01	0.03	16,236	146	1 (reference)	43	1 (reference)	38	1 (reference)	63	1 (reference)
Q2	0.06	0.11	16,882	115	0.74 (0.56-0.97)	36	0.79 (0.50-1.23)	15	0.37 (0.20-0.68)	63	0.94 (0.65-1.36)
Q3	0.15	0.23	16,387	64	0.41 (0.30-0.57)	23	0.51 (0.30-0.85)	13	0.32 (0.17-0.62)	28	0.42 (0.26-0.70)
Q4	0.31	0.39	16,475	90	0.58 (0.43-0.77)	29	0.63 (0.39-1.02)	22	0.54 (0.32-0.94)	39	0.58 (0.38-0.89)
P for trend					<0.001		0.05		0.12		0.002
Continuous, per 0.2 mg/day incr.			65,980	415	0.83 (0.69-0.98)	131	0.86 (0.65-1.12)	88	1.03 (0.72-1.47)	193	0.72 (0.56-0.93)
Supplement vitamin C											
No			58,930	371	1 (reference)	118	1 (reference)	76	1 (reference)	174	1 (reference)
Yes			7,050	44	1.12 (0.80-1.57)	13	0.95 (0.53-1.70)	12	1.48 (0.79-2.75)	19	1.11 (0.68-1.83)
Supplement vitamin E											
No			61,685	392	1 (reference)	124	1 (reference)	82	1 (reference)	183	1 (reference)
Yes			4,296	23	1.01 (0.64-1.58)	7	0.85 (0.39-1.84)	6	1.23 (0.53-2.86)	10	1.05 (0.54-2.05)

[a]OCC: oral cavity cancer; OHPC: oro-/hypopharyngeal cancer; LC: laryngeal cancer. [b]Abbreviations: RR: incidence rate ratio; CI: confidence interval; Q: quartile; incr.: increment.

[c]Nutrient from food intake only. [d]Tests for dose-response trends were assessed by fitting ordinal exposure variables as continuous terms in the Cox proportional hazards model.

[e]The proportional hazards assumption was possibly violated for the exposure variable in this analysis; there was no statistically significant interaction between the exposure variable and time.

[f]The proportional hazards assumption was possibly violated for the exposure variable in this analysis; there was a statistically significant interaction between the exposure variable and time.

Table 4.3. Multivariable-adjusted[a] associations between vitamin and carotenoid intake and risk of head-neck cancer subtypes; Netherlands Cohort Study, 1986–2006

			Subcohort	Head-neck cancer cases							
				Overall		Subtypes					
						OCC[b]		OHPC[b]		LC[b]	
	Median (mg/day)		Person-time	No.	RR[c] (95% CI)	No.	RR (95% CI)	No.	RR (95% CI)	No.	RR (95% CI)
	Men	*Women*	at risk (yrs)	cases		cases		cases		cases	
Vitamin C[d]											
Q1	55.2	63.5	16,281	150	1 (reference)	46	1 (reference)	39	1 (reference)	63	1 (reference)
Q2	79.8	89.2	16,490	101	0.66 (0.48-0.92)	28	0.58 (0.34-0.98)	18	0.40 (0.20-0.79)	54	0.90 (0.58-1.40)
Q3	103.3	113.8	16,661	91	0.58 (0.40-0.83)	36	0.78 (0.45-1.37)[f]	10	0.21 (0.09-0.47)[g]	45	0.74 (0.45-1.23)
Q4	144.8	153.3	16,548	73	0.39 (0.23-0.66)	21	0.35 (0.16-0.77)	21	0.29 (0.12-0.67)	31	0.53 (0.26-1.08)
P for trend[e]					<0.001		0.03		0.003		0.07
Continuous, per 50 mg/day incr.			65,980	415	0.79 (0.58-1.07)[g]	131	0.58 (0.35-0.96)	88	0.72 (0.36-1.46)	193	1.00 (0.68-1.45)
Vitamin E[d]											
Q1	7.6	6.5	16,054	127	1 (reference)	43	1 (reference)	25	1 (reference)	56	1 (reference)
Q2	11.5	9.4	16,588	111	1.05 (0.77-1.43)[f]	38	1.21 (0.74-1.96)	23	1.30 (0.71-2.37)	50	0.95 (0.61-1.47)[f]
Q3	15.7	12.9	16,537	91	0.77 (0.54-1.10)	29	0.82 (0.46-1.47)	20	0.91 (0.43-1.93)[g]	42	0.76 (0.47-1.23)
Q4	22.2	18.4	16,801	86	0.98 (0.67-1.44)[f]	21	0.94 (0.48-1.84)	20	1.63 (0.77-3.47)[g]	45	0.88 (0.52-1.49)[f]
P for trend					0.64[g]		0.61		0.36[g]		0.56[g]
Continuous, per 8.0 mg/day incr.			65,980	415	0.99 (0.81-1.22)	131	1.05 (0.71-1.55)	88	1.22 (0.80-1.84)[g]	193	0.91 (0.70-1.17)[g]
Alpha-carotene[d]											
Q1	0.22	0.20	16,069	124	1 (reference)	43	1 (reference)	20	1 (reference)	58	1 (reference)
Q2	0.45	0.43	16,608	110	1.00 (0.72-1.38)	35	1.03 (0.62-1.73)	27	1.62 (0.84-3.14)	48	0.85 (0.54-1.33)[g]
Q3	0.70	0.68	16,478	93	0.89 (0.60-1.32)	25	0.83 (0.44-1.56)	22	1.39 (0.65-2.98)	46	0.83 (0.48-1.43)[g]
Q4	1.15	1.15	16,824	88	0.88 (0.47-1.65)	28	1.16 (0.46-2.95)	19	1.14 (0.34-3.80)	41	0.72 (0.29-1.74)
P for trend					0.63		0.91		0.88		0.48[g]
Continuous, per 0.5 mg/day incr.			65,980	415	0.78 (0.33-1.84)	131	0.66 (0.14-3.18)	88	0.54 (0.11-2.74)	193	0.98 (0.32-3.06)
Beta-carotene[d]											
Q1	1.57	1.47	16,031	126	1 (reference)	46	1 (reference)	29	1 (reference)	48	1 (reference)
Q2	2.32	2.21	16,615	103	0.85 (0.60-1.20)	33	0.89 (0.51-1.53)	15	0.56 (0.26-1.20)	55	1.05 (0.66-1.67)
Q3	3.06	2.97	16,554	99	0.96 (0.61-1.51)	21	0.76 (0.37-1.55)	27	1.25 (0.49-3.21)	51	1.02 (0.55-1.88)[g]
Q4	4.31	4.27	16,780	87	0.86 (0.41-1.81)	31	1.46 (0.52-4.16)	17	0.71 (0.14-3.68)	39	0.68 (0.25-1.85)
P for trend					0.79		0.63		0.97		0.49

(continued)	Median (mg/day)		Person-time at risk (yrs)	No. cases	RR (95% CI)	No. cases	RR (95% CI)	No. cases	RR (95% CI)	No. cases	RR (95% CI)
Continuous, per 1.5 mg/day incr.			65,980	415	1.35 (0.41-4.52)	131	1.55 (0.18-13.22)	88	2.68 (0.28-25.73)	193	0.97 (0.20-4.72)
Lutein/zeaxanthin[d]											
Q1	1.50	1.38	16,095	124	1 (reference)	44	1 (reference)	24	1 (reference)	54	1 (reference)
Q2	2.05	1.94	16,681	100	0.82 (0.59-1.13)	31	0.73 (0.44-1.24)	29	1.30 (0.70-2.43)	39	0.71 (0.45-1.13)
Q3	2.62	2.52	16,539	89	0.76 (0.51-1.13)	26	0.56 (0.29-1.08)	15	0.66 (0.27-1.58)[g]	48	1.00 (0.60-1.67)
Q4	3.55	3.49	16,665	102	0.85 (0.49-1.47)	30	0.62 (0.27-1.46)	20	1.07 (0.33-3.47)	52	0.98 (0.47-2.06)
P for trend					0.53		0.24		0.85		0.83
Continuous, per 1.0 mg/day incr.			65,980	415	0.96 (0.63-1.46)	131	0.96 (0.46-2.01)	88	0.65 (0.29-1.47)	193	1.10 (0.63-1.94)
Lycopene[d]											
Q1	0.16	0.22	16,141	111	1 (reference)	30	1 (reference)	23	1 (reference)	57	1 (reference)
Q2	0.53	0.69	16,678	97	0.91 (0.66-1.25)	35	1.27 (0.75-2.17)	22	1.00 (0.52-1.91)	40	0.73 (0.46-1.14)
Q3	0.95	1.12	16,687	91	0.87 (0.61-1.23)	26	0.98 (0.55-1.75)	16	0.73 (0.35-1.53)	48	0.91 (0.56-1.46)
Q4	1.70	2.07	16,474	116	0.98 (0.65-1.45)	40	1.30 (0.67-2.52)	27	0.94 (0.41-2.12)	48	0.86 (0.49-1.48)
P for trend					0.98		0.55		0.83		0.79
Continuous, per 0.9 mg/day incr.			65,980	415	1.00 (0.92-1.09)	131	1.07 (0.95-1.21)	88	0.94 (0.79-1.13)	193	0.96 (0.84-1.11)
Beta-cryptoxanthin[d]											
Q1	0.01	0.03	16,236	146	1 (reference)	43	1 (reference)	38	1 (reference)	63	1 (reference)
Q2	0.06	0.11	16,882	115	0.84 (0.62-1.15)	36	0.93 (0.56-1.54)	15	0.35 (0.17-0.71)	63	1.12 (0.74-1.69)
Q3	0.15	0.23	16,387	64	0.47 (0.32-0.69)	23	0.62 (0.33-1.16)	13	0.28 (0.13-0.62)	28	0.52 (0.30-0.89)
Q4	0.31	0.39	16,475	90	0.73 (0.47-1.14)	29	1.08 (0.51-2.27)	22	0.47 (0.21-1.06)	39	0.76 (0.42-1.40)
P for trend					0.05		0.93		0.11		0.08
Continuous, per 0.2 mg/day incr.			65,980	415	1.08 (0.81-1.43)[g]	131	1.38 (0.84-2.29)	88	1.49 (0.80-2.77)	193	0.79 (0.57-1.10)
Supplement vitamin C											
No			58,930	371	1 (reference)	118	1 (reference)	76	1 (reference)	174	1 (reference)
Yes			7,050	44	1.18 (0.76-1.85)	13	0.99 (0.53-1.86)	12	1.53 (0.66-3.59)	19	1.21 (0.65-2.24)
Supplement vitamin E											
No			61,685	392	1 (reference)	124	1 (reference)	82	1 (reference)	183	1 (reference)
Yes			4,296	23	0.96 (0.54-1.70)	7	0.90 (0.40-2.03)	6	0.87 (0.28-2.72)	10	1.07 (0.47-2.43)

Table 4.3 (footnotes).

[a]Mutually adjusted for age (years); sex; cigarette smoking (status (never/former/current), frequency (number of cigarettes per day; continuous, centered), duration (number of years; continuous, centered)); alcohol consumption (grams ethanol per day; continuous); and total daily energy intake (kcal/day; continuous).

[b]OCC: oral cavity cancer; OHPC: oro-/hypopharyngeal cancer; LC: laryngeal cancer.

[c]Abbreviations: RR: incidence rate ratio; CI: confidence interval; Q: quartile; incr.: increment.

[d]Nutrient from food intake only.

[e]Tests for dose-response trends were assessed by fitting ordinal exposure variables as continuous terms in the Cox proportional hazards model.

[f]The proportional hazards assumption was possibly violated for the exposure variable in this analysis; there was a statistically significant interaction between the exposure variable and time.

[g]The proportional hazards assumption was possibly violated for the exposure variable in this analysis; there was no statistically significant interaction between the exposure variable and time.

Table 4.4. Stratified analyses of sex/cigarette smoking/alcohol consumption and quartiles of vitamin C intake and risk (multivariable-adjusted[a] associations) of head-neck cancer overall; Netherlands Cohort Study, 1986–2006

	Head-neck cancer overall			
	Vitamin C intake			
	Q1[b]	Q2	Q3	Q4
Sex				
M				
Cases/person-time at risk (years)	110/7,373	84/7,747	72/7,868	54/7,682
RR (95% CI)	1 (ref)	0.75 (0.52-1.10)	0.63 (0.42-0.94)	0.40 (0.22-0.71)
F				
Cases/person-time at risk (years)	40/8,909	17/8,743	19/8,793	19/8,867
RR (95% CI)	0.54 (0.34-0.86)	0.22 (0.12-0.41)	0.23 (0.13-0.42)	0.19 (0.10-0.38)
P for interaction[c]	0.34			
Cigarette smoking				
Never				
Cases/person-time at risk (years)	19/6,130	10/6,595	15/6,080	12/7,029
RR (95% CI)	1 (reference)	0.45 (0.21-1.00)	0.71 (0.34-1.47)	0.37 (0.15-0.90)
Former				
Cases/person-time at risk (years)	36/5,320	27/5,889	33/6,386	29/6,127
RR (95% CI)	1.25 (0.67-2.31)	0.79 (0.41-1.53)	0.85 (0.44-1.64)	0.64 (0.30-1.34)
Current				
Cases/person-time at risk (years)	95/4,832	64/4,006	43/4,195	32/3,392
RR (95% CI)	2.71 (1.53-4.80)	2.02 (1.11-3.66)	1.34 (0.71-2.53)	0.93 (0.44-1.98)
P for interaction	0.53			
Alcohol consumption				
Abstainers				
Cases/person-time at risk (years)	14/4,382	10/3,768	5/3,449	12/3,810
RR (95% CI)	0.62 (0.32-1.19)	0.56 (0.27-1.16)	0.25 (0.09-0.67)	0.45 (0.21-0.97)
>0-15 (g/day)				
Cases/person-time at risk (years)	48/8,223	37/8,923	46/8,852	28/8,415
RR (95% CI)	1 (reference)	0.68 (0.42-1.10)	0.89 (0.55-1.44)	0.50 (0.26-0.98)
≥15 (g/day)				
Cases/person-time at risk (years)	88/3,677	54/3,799	40/4,360	33/4,323
RR (95% CI)	2.16 (1.42-3.28)	1.42 (0.89-2.25)	0.87 (0.52-1.45)	0.69 (0.38-1.24)
P for interaction	0.08			

[a]Mutually adjusted for age (years); sex; cigarette smoking (status (never/former/current), frequency (number of cigarettes per day; continuous, centered), duration (number of years; continuous, centered)); alcohol consumption (grams ethanol per day; continuous); and total daily energy intake (kcal/day; continuous).

[b]Abbreviations: RR: incidence rate ratio; CI: confidence interval; Q: quartile.

[c]*P* value for interaction based on cross-product terms in the Cox proportional hazards model and Wald test.

Table 4.5. Stratified analyses of sex/cigarette smoking/alcohol consumption and quartiles of vitamin E intake and risk (multivariable-adjusted[a] associations) of head-neck cancer overall; Netherlands Cohort Study, 1986–2006

	Head-neck cancer overall			
	Vitamin E intake			
	Q1[b]	Q2	Q3	Q4
Sex				
M				
Cases/person-time at risk (years)	91/7,413	84/7,733	73/7,628	72/7,896
RR (95% CI)	1 (reference)	1.12 (0.78-1.61)	0.87 (0.57-1.32)	1.16 (0.75-1.80)
F				
Cases/person-time at risk (years)	36/8,641	27/8,856	18/8,909	14/8,905
RR (95% CI)	0.60 (0.38-0.95)	0.54 (0.33-0.90)	0.35 (0.19-0.62)	0.34 (0.18-0.64)
P for interaction[c]	0.27			
Cigarette smoking				
Never				
Cases/person-time at risk (years)	23/5,996	15/6,541	9/6,325	9/6,972
RR (95% CI)	1 (reference)	0.67 (0.34-1.34)	0.43 (0.19-0.96)	0.44 (0.20-0.98)
Former				
Cases/person-time at risk (years)	26/5,666	33/6,112	32/5,921	34/6,023
RR (95% CI)	0.63 (0.33-1.18)	0.99 (0.54-1.81)	0.94 (0.51-1.74)	1.17 (0.62-2.20)
Current				
Cases/person-time at risk (years)	78/4,391	63/3,936	50/4,290	43/3,806
RR (95% CI)	2.04 (1.18-3.53)	2.03 (1.14-3.63)	1.34 (0.71-2.51)	1.76 (0.92-3.35)
P for interaction	0.06			
Alcohol consumption				
Abstainers				
Cases/person-time at risk (years)	15/4,497	20/3,968	4/3,313	2/3,630
RR (95% CI)	0.62 (0.32-1.18)	1.42 (0.77-2.61)	0.29 (0.10-0.85)	0.15 (0.03-0.64)
>0-15 (g/day)				
Cases/person-time at risk (years)	37/7,414	37/8,751	37/8,918	48/9,330
RR (95% CI)	1 (reference)	0.86 (0.52-1.40)	0.91 (0.55-1.50)	1.23 (0.74-2.05)
≥15 (g/day)				
Cases/person-time at risk (years)	75/4,143	54/3,870	50/4,305	36/3,841
RR (95% CI)	1.97 (1.26-3.09)	1.77 (1.09-2.88)	1.46 (0.87-2.45)	1.26 (0.71-2.24)
P for interaction	0.003			

[a]Mutually adjusted for age (years); sex; cigarette smoking (status (never/former/current), frequency (number of cigarettes per day; continuous, centered), duration (number of years; continuous, centered)); alcohol consumption (grams ethanol per day; continuous); and total daily energy intake (kcal/day; continuous).

[b]Abbreviations: RR: incidence rate ratio; CI: confidence interval; Q: quartile.

[c]*P* value for interaction based on cross-product terms in the Cox proportional hazards model and Wald test.

Discussion

This study examined associations of vitamin and carotenoid intake with HNC risk. Strong inverse associations were found between vitamin C and HNC overall, OCC, and OHPC. No clear associations were found for other nutrients and vitamin supplementation; however, most point estimates showed possible protective effects. In addition, no clear differences were found between the associations of vitamin and carotenoid intake and the risk on subtypes of HNC. Furthermore, the association between vitamin E intake and the risk on HNC overall was modified by alcohol consumption.

Vitamin and carotenoid intake

To our knowledge, this is the largest prospective study to investigate the association of vitamin and carotenoid intake and the risk on HNC, including the most common subtypes OCC, OHCP, and LC. The Iowa Women's Health Study (IWHS) reported a relative risk of 0.7 (95% CI 0.3-1.7) for vitamin C, comparing tertile 3 vs. tertile 1.[17] A stronger effect is found in our study, possibly due to the fact that quartiles result in more contrast between the groups compared with tertiles. A nested case-control study suggested a protective effect of carotenoid intake on oral cancer. However, only 28 cases were diagnosed with primary oral and pharyngeal cancer.[32] Thus, evidence from prospective cohort studies is scarce, but supports our results.

The INHANCE consortium suggested that vitamin C supplementation reduces the risk of HNC, but no linear trend was observed.[33] A pooled analysis provided the same results as our study; no clear associations were found between supplementation and the risk on HNC.[34] The different associations of vitamin supplements and vitamin intake from food could be explained by the small sample size of supplement users. Furthermore, vitamin supplements could have other properties than vitamin intake through food.

A number of case control studies investigated vitamin and carotenoid intake and the risk on HNC.[35-38] A case-control report of Negri et al.[37] reported an odds ratio (OR) of 0.34 (95% CI 0.23-0.51) for vitamin C comparing quintile 5 vs. quintile 1 and oral and pharyngeal cancer. Furthermore, Negri et al. found significant protective associations for vitamin E (OR: 0.44, 95% CI 0.28-0.71) and carotene (OR: 0.43, 95% CI 0.28-0.66) intake, comparing the highest quintile with the lowest quintile.[37] The differences with our study could be explained by the different study design, since recall bias, selection bias and reversed causation are more likely to occur in a case-control design or

because of hospital-based controls, which may not be representative of the general population.

Overall, vitamin C is suggested to have a stronger inverse association with HNC than with other micronutrients. However, epidemiological evidence for other micro-nutrients remains inconclusive. In addition, evidence from case-control studies regarding the micro-nutrients and HNC risk provides inconsistent results.

The association of vitamin C intake in our study seems to be stronger for OCC compared with OHPC and LC. The results of other studies are inconsistent, and therefore, no conclusion could be drawn. However, because the intake of carotenoids and vitamins is highly dependent on the intake of fruit and vegetables[11], results could be compared to associations of fruit and vegetable intake. A large prospective cohort study for the association between fruit and vegetables intake and HNC subtypes confirms that there is a stronger benefit for OCC and OHPC with a high intake, compared with LC.[10,12]

Interaction between alcohol and smoking and intake of vitamins and carotenoids

No clear differences in associations with HNC were found among strata of cigarette smoking. The INHANCE consortium[33] showed no significant associations for supplement use of vitamin C or E when stratified by smoking or drinking status. However, due to the small number of subjects in that group and therefore a lack of power, conclusions cannot be drawn. We found differences in the risk estimates among strata of smoking and alcohol use. It is plausible that alcohol consumption and smoking status would modify the association between the intake of vitamins and carotenoids and HNC. Oxidative stress could be caused by tobacco or alcohol use, which can be prevented by the effect of vitamins.[39,40]

Thus, only one other study investigated interaction effects of cigarette smoking and alcohol consumption.[33] Because of low number of participants in the strata, evidence about potential interaction effects on HNC risk remains inconclusive.

Mechanisms

The exact mechanisms of vitamins and carotenoids with regard to cancer risk are not fully known.[41] It is suggested that they play a protective role in cancer and cardiovascular diseases.[42-44] Deficiency of micronutrients can damage DNA[45] and vitamin C might play a role in the chemoprevention of cancer; the micronutrient blocks metabolic activation of carcinogens, prevents oxidative stress and stimulates the immune function.[39] Furthermore, vitamin E helps to detoxify drugs and other toxins

that promote the development of oxidative stress.[40] Vitamin A, which is produced by alpha- and beta-carotene, induces differentiation and apoptosis.[46]

Differences in associations among HNC subtypes could be explained by different exposures due to the location of the tumor, as supposed by the WCRF.[2] The mouth and pharynx are directly exposed to vitamin and carotenoid intake, which could lead to stronger inverse effects, compared with cancer of the larynx.

In conclusion, there is evidence about the protective effect for micronutrients regarding cancer; however, this evidence remains inconclusive and needs more clarification.

Strengths and limitations

An important strength of the NLCS is the prospective design, in which exposures were assessed before diagnosis. Therefore, it is not likely that recall bias or reversed causation influenced the results, which is confirmed by sensitivity analyses. Second, the NLCS makes it possible to distinguish between the most common HNC subtypes, due to the large number of participants. In addition, effect modification of alcohol consumption and smoking status could be investigated. Besides, tobacco smoking and alcohol use are elaborately measured in the questionnaire, which makes the occurrence of residual confounding for these variables unlikely. This is also confirmed by comparing the age- and sex-adjusted analyses with the multivariable-adjusted analyses. Furthermore, it is unlikely that loss to follow-up bias occurred, because of the high completeness of follow-up of both subcohort members and cases.

There are some limitations to this study. First, misclassification could have occurred for the exposure variables. If so, we expect it to be non-differential and could cause an underestimation of the effects. However, the participants had steady diet habits and the validation and reproducibility study showed that vitamins and carotenoids could rank subjects properly according to intake of the nutrients.[24,27] Second, literature has shown that HPV is associated with an increased HNC risk.[5] The questionnaire did not provide any information about HPV infections of the participants. This could have led to biased results. Third, proportional hazard assumptions were not fulfilled for some analyses. However, because of the limited number of cases we could not investigate this into depth.

Conclusions

This large and long-term prospective cohort study shows an inverse association between intake of vitamin C and the incidence of HNC (subtypes) and indicates an inverse association between the intake of vitamin E and carotenoids and the incidence

of HNC and HNC subtypes. Future research is recommended to confirm our results and further investigate the underlying mechanisms, which may be promising for the prevention of HNC.

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Supplemental Table 4.1. Sensitivity analyses (first two years of follow-up excluded): multivariable-adjusted[a] associations between vitamin and carotenoid intake and risk of head-neck cancer (HNC) overall; Netherlands Cohort Study, 1986–2006

	Subcohort			HNC overall	
	Median (mg/day)		Person-time	No. of	RR[b] (95% CI)
	Men	*Women*	at risk (yrs)	cases	
Vitamin C[c]					
Q1	55.2	63.5	14,356	137	1 (reference)
Q2	79.8	89.2	14,557	93	0.66 (0.47-0.93)
Q3	103.3	113.8	14,727	83	0.57 (0.39-0.83)
Q4	144.8	153.3	14,613	69	0.39 (0.23-0.68)
P for trend[d]					0.001
Continuous, per 50 mg/day increment			58,253	382	0.73 (0.53-1.00)
Vitamin E[c]					
Q1	7.6	6.5	14,125	119	1 (reference)
Q2	11.5	9.4	14,655	100	1.01 (0.73-1.40)[e]
Q3	15.7	12.9	14,597	87	0.80 (0.55-1.14)
Q4	22.2	18.4	14,876	76	0.92 (0.62-1.37)[e]
P for trend					0.50[e]
Continuous, per 8.0 mg/day increment			58,253	382	0.98 (0.79-1.20)
Alpha-carotene[c]					
Q1	0.22	0.20	14,143	112	1 (reference)
Q2	0.45	0.43	14,670	101	1.02 (0.72-1.42)
Q3	0.70	0.68	14,551	86	0.92 (0.61-1.39)
Q4	1.15	1.15	14,888	83	0.93 (0.48-1.81)
P for trend					0.77
Continuous, per 0.5 mg/day increment			58,253	382	0.82 (0.33-2.02)
Beta-carotene[c]					
Q1	1.57	1.47	14,104	114	1 (reference)
Q2	2.32	2.21	14,680	94	0.86 (0.60-1.24)
Q3	3.06	2.97	14,620	93	1.01 (0.63-1.62)
Q4	4.31	4.27	14,849	81	0.90 (0.41-1.98)
P for trend					0.91
Continuous, per 1.5 mg/day increment			58,253	382	1.28 (0.36-4.52)
Lutein/zeaxanthin[c]					
Q1	1.50	1.38	14,169	113	1 (reference)
Q2	2.05	1.94	14,742	92	0.84 (0.60-1.17)
Q3	2.62	2.52	14,610	83	0.80 (0.53-1.21)
Q4	3.55	3.49	14,733	94	0.91 (0.51-1.61)
P for trend					0.73
Continuous, per 1.0 mg/day increment			58,253	382	0.98 (0.63-1.53)
Lycopene[c]					
Q1	0.16	0.22	14,208	101	1 (reference)
Q2	0.53	0.69	14,738	92	0.94 (0.67-1.31)
Q3	0.95	1.12	14,758	81	0.84 (0.58-1.21)
Q4	1.70	2.07	14,549	108	0.99 (0.66-1.50)
P for trend					1.00
Continuous, per 0.9 mg/day increment			58,253	382	1.01 (0.93-1.11)
Beta-cryptoxanthin[c]					
Q1	0.01	0.03	14,307	133	1 (reference)
Q2	0.06	0.11	14,947	103	0.83 (0.60-1.15)
Q3	0.15	0.23	14,452	62	0.51 (0.34-0.76)
Q4	0.31	0.39	14,547	84	0.79 (0.50-1.25)
P for trend					0.14

(continued)	Subcohort			HNC overall	
	Median (mg/day)		Person-time	No. of	RR (95% CI)
	Men	*Women*	at risk (yrs)	cases	
Continuous, per 0.2 mg/day increment			58,253	382	1.15 (0.86-1.55)
Supplement vitamin C					
No			52,020	341	1 (reference)
Yes			6,233	41	1.21 (0.76-1.92)
Supplement vitamin E					
No			54,457	361	1 (reference)
Yes			3,796	21	0.93 (0.51-1.69)

[a]Mutually adjusted for age (years); sex; cigarette smoking (status (never/former/current), frequency (number of cigarettes per day; continuous, centered), duration (number of years; continuous, centered)); alcohol consumption (grams ethanol per day; continuous); and total daily energy intake (kcal/day; continuous).

[b]Abbreviations: RR: incidence rate ratio; CI: confidence interval; Q: quartile.

[c]Nutrient from food intake only.

[d]Tests for dose-response trends were assessed by fitting ordinal exposure variables as continuous terms in the Cox proportional hazards model.

[e]The proportional hazards assumption was possibly violated for the exposure variable in this analysis; there was no statistically significant interaction between the exposure variable and time.

Chapter 5

Toenail selenium status and risk of subtypes of head-neck cancer: the Netherlands Cohort Study

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Abstract

Background

There is limited prospective data on the relationship between selenium status and the risk of head-neck cancer (HNC) and HNC subtypes (i.e., oral cavity cancer (OCC), oro-/hypopharyngeal cancer (OHPC), and laryngeal cancer (LC)). Therefore, we investigated the association between toenail selenium, reflecting long-term selenium exposure, and HNC risk within the Netherlands Cohort Study.

Methods

At baseline, 120,852 participants completed a self-administered questionnaire about diet and other cancer risk factors and were asked to provide toenail clippings. After 20.3 years of follow-up, 294 cases of HNC (95 OCC, 62 OHPC, two oral cavity/pharynx unspecified or overlapping, and 135 LC) and 2,164 subcohort members were available for case-cohort analysis using Cox proportional hazards models.

Results

Toenail selenium status was statistically significantly associated with a decreased risk of HNC overall (multivariable-adjusted incidence rate ratio for quartile four vs. one: 0.55, 95% confidence interval 0.37-0.82, *P* trend=0.001). The association between toenail selenium and risk of HNC overall was stronger among men than women, but no statistically significant interaction with sex was found. Toenail selenium level was also associated with a decreased risk of all HNC subtypes, with statistically significant associations in OHPC and LC. No statistically significant interaction was found between toenail selenium level and cigarette smoking or alcohol consumption for HNC overall.

Conclusions

In this large cohort study, we found an inverse association between toenail selenium level and HNC risk. Among HNC subtypes, this association was strongest for OHPC and LC. Furthermore, the association of toenail selenium status with HNC risk was stronger among men than women.

Introduction

Selenium is a trace element present in food which is likely to be involved in cancer risk. It might act in various stages of tumor development and in several anticarcinogenic processes.[1,2] Selenium functions as an essential component of selenoproteins, antioxidant enzymes—including glutathione peroxidases—that protect against oxidative damage. When selenium is incorporated into these selenoproteins, they may influence carcinogenesis by their antioxidant properties.[1,2] Other mechanisms regarding the anticancer effect of selenium include alteration of DNA methylation, cell cycle regulation, enhancement of immune function, and inhibition of angiogenesis.[1,2]

Evidence for an effect of selenium on cancer risk has been reviewed and includes findings from different types of study, including in vitro, animal, and ecological studies.[1] In observational studies, selenium status has been associated with a decreased risk of prostate and esophageal cancer and possibly also of lung and stomach cancer.[3-5]

A low selenium status may also be associated with an increased risk of head and neck cancer (HNC). HNC is the seventh most common type of cancer in the world and alcohol consumption, cigarette smoking, and human papillomavirus (HPV) infection are established risk factors for HNC.[5,6] Evidence regarding selenium and HNC risk is, however, scarce.

In a population-based case-control study in the United States, low toenail selenium status was associated with an increased risk of oral cancer, but only in men.[7] Two nested case-control studies in Finland and the United States yielded mixed results with respect to the association of serum selenium status and the risk of HNC, but the number of cases in both studies was very small.[8,9]

Selenium status can be measured in various ways. Worldwide, the dietary intake of selenium varies considerably, with a relatively high intake in the United States compared to Europe, as a result of differences in selenium soil content.[2] Consequently, selenium levels in food vary such that the measurement of selenium intake through food questionnaires is flawed. Blood selenium reflects selenium exposure over recent weeks, whereas toenail selenium reflects the intake of selenium for a period up to one year.[10] Furthermore, given the leading role of smoking and alcohol consumption in the development of HNC, adequate adjustment for confounding by these factors is of great importance when investigating selenium and HNC risk.

We investigated the association between toenail selenium levels and total HNC and HNC subtypes within the large prospective Netherlands Cohort Study (NLCS). We focused on the most frequent HNC subtypes[11] (those located in the oral cavity,

pharynx, and larynx) and hypothesized that the risk of HNC is higher in participants with low levels of toenail selenium. In addition, we examined possible effect modification of the association between toenail selenium and HNC risk by sex, cigarette smoking, or alcohol consumption.

Methods

Study design and population

The present study was conducted within the NLCS, which was initiated in September 1986 and includes 120,852 participants, aged 55-69 years from 204 Dutch municipal population registries.[12] At baseline, all participants completed a self-administered questionnaire about diet, lifestyle habits, and other cancer risk factors. In addition, they were asked to provide toenail clippings; approximately 75% of the subjects provided these. The NLCS has been approved by the Medical Ethics Committee of Maastricht University (Maastricht, The Netherlands). Participants were informed that by returning the questionnaire and toenail sample they would give their consent to participate in a study of the etiology of cancer.

We used the case-cohort design for efficiency in data processing and follow-up.[13] Cases were derived from the entire cohort, whereas the number of person-years at risk for the entire cohort was estimated from a subcohort of 3,500 people who were randomly sampled from the total cohort at baseline.

Follow-up for cancer incidence was done by record linkage to the Netherlands Cancer Registry (NCR) and the nationwide network and pathology registry (PALGA).[14] Follow-up for vital status of the subcohort was nearly 100% complete after 20.3 years and the completeness of cancer follow-up is estimated to be ≥96%.[15]

Only microscopically confirmed, first occurrences of squamous cell carcinomas—which include nearly all malignancies of the mouth, pharynx, and larynx—were included in our study (Figure 5.1).[5,16] We excluded cohort members who reported having prevalent cancer other than skin cancer at baseline. In addition, we excluded participants who had incomplete/inconsistent dietary data, did not provide a toenail sample, had a sample with a too low sample weight (<10mg), or had missing data on confounding variables (see below).[17]

Complete data for statistical analysis with toenail selenium were available for 2,164 subcohort members and 294 incident cases of the selected HNC subtypes (Figure 5.1). HNC subtypes were classified as proposed by Hashibe et al.[18] according to the International Classification of Diseases for Oncology[19] (Table 5.1).

Table 5.1. Subclassification of subtypes of head-neck cancer (HNC) as proposed by Hashibe et al.[18], according to the International Classification of Diseases for Oncology, version 3 (ICD-O-3).[19]

HNC-subtype	ICD-O-3
Oral cavity cancer (OCC)	C003-009, C020-C023, C030-C031, C039-C041, C048-C050, C060-C062, C068-C069
Oro-/hypopharyngeal cancer (OHPC)	C019, C024, C051-C052, C090-C091, C098-C104, C108-C109, C129-C132, C138-C139
Oral cavity, pharynx unspecified or overlapping cancer (USC)	C028-C029, C058-C059, C140-C142, C148
Laryngeal cancer (LC)	C320-C329

Of the 294 HNC cases, 95 were oral cavity cancer (OCC), 62 were oro-/hypopharyngeal cancer (OHPC), two were oral cavity/pharynx unspecified or overlapping (only included in analyses of HNC overall), and 135 were laryngeal cancer (LC) cases.

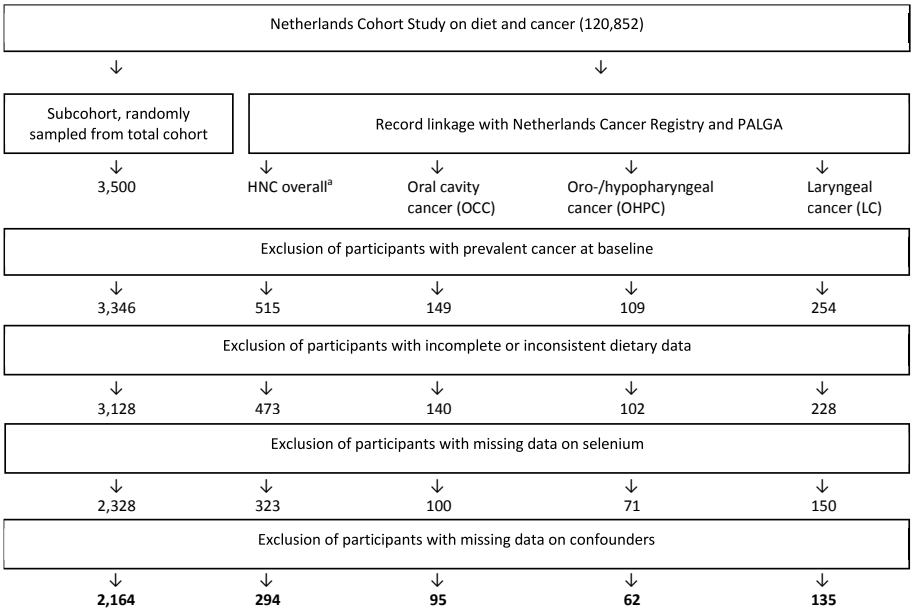

Figure 5.1. Flow diagram of the number of subcohort members and cases on whom the analyses were based.
Abbreviation PALGA: nationwide network and registry of histopathology and cytopathology in the Netherlands.
[a]Oral cavity cancer; oro-/hypopharyngeal cancer; oral cavity, pharynx unspecified or overlapping cancer; laryngeal cancer.

Exposure data

Selenium concentrations were measured in toenail clippings by the Reactor Institute Delft (Delft University of Technology; Delft, The Netherlands), using instrumental neutron activation analysis of the ^{77m}Se isotope (metastable selenium-77 isotope, half-life 17.5 seconds). Each sample went through six cycles of 17-second irradiation at a thermal neutron flux of $3*10^{16}$ $m^{-2}s^{-1}$, 3-second decay, and 17-second counting at 1 cm

from a 40% germanium detector. The accuracy of the neutron activation analysis was checked by analysis of a certified bovine liver standard (Standard Reference Material 1577b, later replaced by 1577c, of the United States National Institute of Standards and Technology). This method and the NLCS toenail selenium project have been described in more detail previously.[4,20,21]

The toenail selenium measurements for the subcohort were carried out in 1992. In 2012-2013, toenail selenium levels of HNC cases diagnosed during 20.3 years of follow-up were measured. In 1992, the 'Snelle Buizen Post' (SBP) facility was used for instrumental neutron activation analysis, and, since 1996, the 'Carbonfiber Autonomous Facility for Irradiation and Analysis' (CAFIA) facility is used. To assess the validity and comparability of these two methods, toenail selenium levels of the same 40 subcohort members were determined in 1996 with the 'CAFIA' facility in addition to the original assessment with the 'SBP' facility.[22] The mean selenium level (μg/g) assessed by the 'CAFIA' facility (0.552; standard deviation (SD): 0.05) was similar to the mean selenium level (μg/g) assessed by the 'SBP' facility (0.551; SD: 0.04), with a Pearson correlation coefficient of 0.95 (P <0.01).[22] It was concluded that both methods were valid and comparable.

The questionnaire included a 150-item food frequency questionnaire that focused on habitual food consumption during the year preceding the start of the study. In addition, we asked questions about lifestyle habits and other cancer risk factors. We asked detailed questions about alcohol consumption and cigarette smoking, as described before.[23] 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.

Statistical analysis

The Cox proportional hazards model was used to estimate age- and sex-adjusted and multivariable-adjusted incidence rate ratios (RR) and corresponding 95% confidence intervals (CI). Person-years at risk were calculated from baseline until diagnosis of HNC, death, emigration, loss to follow-up, or end of follow-up, whichever occurred first. For analyses of HNC overall, we categorized toenail selenium levels into quartiles according to the sex-specific distribution in the subcohort. For analyses of HNC subtypes, we decided to categorize toenail selenium levels into sex-specific tertiles because of the limited number of cases. For continuous analyses, selenium levels were standardized to the average size of the two central quartiles in the subcohort; one standardized selenium unit equals 0.08 μg/g.

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. We compared the mean selenium level of HNC cases diagnosed during the first two years of follow-up with the level of cases diagnosed later in follow-up. In addition, after we applied a natural logarithm (ln)-transformation to normalize the distribution of selenium levels, we used an independent samples t-test to test the statistical significance of differences. Based on these results, we decided to include the total follow-up time in our analyses. We also performed sensitivity analyses regarding the association between toenail selenium status and the risk of HNC overall by excluding the first two years of follow-up.

The predefined confounders were age (years); sex; alcohol consumption (grams/day); and cigarette smoking (status (never/former/current), number of cigarettes smoked daily, and number of smoking years). We considered the following potential confounders: level of education; body mass index; non-occupational physical activity; intake of vegetables and fruits, fish, red meat, and meat products (all grams/day); and family history of HNC.[5,24] None of these variables changed the RR for toenail selenium (continuous) for HNC overall or any of the HNC subtypes by >10% when including them in the model. Therefore, the final model included only the predefined confounders. When adjusting for cigarette smoking frequency and duration, we centered these continuous variables as proposed by Leffondré et al.[25]

We assessed tests for linear dose-response trends by fitting ordinal exposure variables as continuous terms. Standard errors were estimated using the robust Huber-White sandwich estimator to account for additional variance due to sampling from the cohort. The proportional hazards (PH) assumption was assessed using the scaled Schoenfeld residuals.[26] If there was an indication for violation of the PH assumption for a variable, it was further investigated by adding a time-varying covariate for that variable to the model. We performed analyses for HNC and all HNC subtypes using a time-varying covariate for current smoking, as described before.[27]

To determine whether sex, cigarette smoking, or alcohol consumption possibly modify the association of toenail selenium status (both on a categorical and continuous scale) with risk of HNC overall, we estimated RRs in strata of these exposures. Tests for interaction were performed with toenail selenium levels on a categorical (quartiles) and continuous scale. *P* values for interaction were assessed by including cross-product terms in the models and performing a Wald test.

No statistically significant interaction with sex was found. However, we noticed considerable differences in risk estimates for HNC overall between men and women. Therefore (and because of relatively few female cases in HNC subtypes), analyses were

carried out for both sexes combined and, for HNC overall, also for men and women separately. We performed analyses in strata of alcohol consumption (abstainers; consuming >0 to 15 grams ethanol/day; consuming ≥15 grams ethanol/day) and cigarette smoking status (never/former/current). Alcohol consumption and cigarette smoking were mutually adjusted in these models.

All reported *P* values were based on two-sided tests and considered statistically significant if <0.05. Analyses were done using the Stata 11.2 statistical software package (StataCorp, College Station, Texas, USA).

Results

Baseline characteristics

Mean toenail selenium levels were considerably lower among cases (0.517 μg/g) than subcohort members (0.562 μg/g) (Table 5.2). Compared with subcohort members, cases were far more often men than women. Furthermore, among subcohort members, men had noticeably lower toenail selenium levels (0.549 μg/g) than women (0.575 μg/g). There were also far more current smokers among cases than subcohort members, and the frequency and duration of cigarette smoking were higher among cases. Finally, cases were less frequently alcohol abstainers and had a substantially higher alcohol intake than subcohort members.

There was no clear trend toward higher or lower toenail selenium levels in the HNC cases in the course of 20.3 years of follow-up (Table 5.3). HNC overall cases diagnosed after the second year of follow-up (*N*=269) had a mean toenail selenium level of 0.519 μg/g; cases diagnosed during the first two years of follow-up (*N*=25) had a mean toenail selenium level of 0.494 μg/g. This difference was not statistically significant.

Main analyses

Results from age- and sex-adjusted and multivariable-adjusted analyses both showed mostly inverse associations between toenail selenium level and HNC risk, but most associations were substantially stronger in age- and sex-adjusted analyses, especially in HNC subtypes (Tables 5.4 and 5.5).

Toenail selenium status was statistically significantly associated with a decreased risk of HNC overall, on a categorical (multivariable-adjusted RR for quartile four vs. one: 0.55, 95% CI 0.37-0.82, P trend=0.001) and continuous scale (multivariable-adjusted RR per increment in standard selenium unit: 0.82, 95% CI 0.73-0.91) (Table

5.4). The association between toenail selenium and risk of HNC overall was considerably stronger among men (RR: 0.46, 95% CI 0.28-0.76 for quartile four vs. one, P trend<0.001) than women (RR: 0.89, 95% CI 0.44-1.83, P trend=0.63), but no statistically significant interaction with sex was found (P=0.28 (continuous scale) and 0.43 (categorical)). Sensitivity analyses showed essentially similar results after exclusion of the first two years of follow-up.

Among HNC subtypes, toenail selenium level was associated with a decreased risk of HNC, with statistically significant associations in OHPC (multivariable-adjusted RR per increment in standard selenium unit: 0.72, 95% CI 0.58-0.90) and LC (RR per standard unit: 0.83, 95% CI 0.71-0.96) but not OCC (RR per standard unit: 0.88, 95% CI 0.75-1.04) (Table 5.5). For all subtypes, decreased risks were also found across tertiles of toenail selenium, but without statistical significance. The inverse associations between toenail selenium and HNC risk were generally strongest for OHPC.

Stratified analyses

No statistically significant interaction was found between quartiles of toenail selenium level and either cigarette smoking (*P* interaction=0.94) or alcohol consumption (*P* interaction=0.32) for HNC overall (Table 5.6). Within strata of cigarette smoking, we found largely inverse associations between toenail selenium and HNC; we found a statistically significantly decreased risk of HNC overall for current smokers in the highest compared with the lowest quartile. Stratified analyses with toenail selenium on a continuous scale showed the same pattern. Within strata of alcohol consumption, we found mostly inverse associations between toenail selenium and HNC risk in all strata as well (Table 5.6).

Table 5.2. Characteristics of cases and subcohort members; Netherlands Cohort Study, 1986–2006

	Subcohort	Head-neck cancer cases			
		Overall	Subtypes		
			OCC[a]	OHPC[a]	LC[a]
Exposure variables and confounders[b]	**(*N*=2,164)[c]**	**(*N*=294)[c]**	**(*N*=95)[c]**	**(*N*=62)[c]**	**(*N*=135)[c]**
Toenail selenium level (µg/g), mean (SD)					
Total	0.562 (0.118)	0.517 (0.089)	0.536 (0.093)	0.503 (0.092)	0.511 (0.083)
Men	0.549 (0.125)	0.506 (0.087)	0.517 (0.099)	0.492 (0.080)	0.508 (0.082)
Women	0.575 (0.109)	0.555 (0.087)	0.561 (0.078)	0.537 (0.115)	0.562 (0.079)
Age at baseline (years)	61.3 (4.2)	61.7 (4.1)	61.7 (4.3)	61.7 (4.1)	61.6 (4.0)
Sex: men (%)	49.0	77.2	56.8	74.2	93.3
Cigarette smoking status (%)					
Never smoker	38.0	15.0	32.6	8.1	5.9
Former smoker	36.7	30.6	25.3	30.7	34.8
Current smoker	25.3	54.4	42.1	61.3	59.3
Ever cigarette smokers:					
Frequency of cigarette smoking (*N*/day)	15.1 (10.2)	19.1 (11.1)	20.8 (11.6)	21.5 (13.2)	17.1 (9.5)
Duration of cigarette smoking (years)	31.1 (12.3)	38.5 (9.8)	37.0 (9.9)	37.6 (9.7)	39.5 (9.8)
Pack-years of cigarette smoking (*N*)	22.1 (17.4)	33.0 (20.6)	35.7 (23.3)	36.0 (22.4)	30.3 (18.2)
Abstainer from alcohol (%)	24.0	9.5	10.5	12.9	7.4
Alcohol consumers: ethanol intake (g/day)	13.3 (15.0)	27.0 (25.7)	26.3 (28.4)	36.4 (32.2)	23.2 (19.0)

[a]OCC: oral cavity cancer; OHPC: oro-/hypopharyngeal cancer; LC: laryngeal cancer.

[b]Values are given as mean (SD); for categorical variables, *N* (%) is presented.

[c]The number of subcohort members or cases (with complete data on toenail selenium level, age, sex, cigarette smoking, and alcohol consumption) used in analyses of toenail selenium.

Table 5.3. Toenail selenium levels in head-neck cancer (HNC) cases according to sex and time between baseline and HNC diagnosis; Netherlands Cohort Study, 1986–2006

	Head-neck cancer overall			
		Toenail selenium level (µg/g)		
Cases[a]	**No. of cases**	**Mean**	**SD**	***P* value**
All cases	294	0.517	0.089	
Men	227	0.506	0.087	
Women	67	0.555	0.087	
Year of follow-up				
0-2	25	0.494	0.098	
>2	269	0.519	0.088	0.13[b]
Categories year of follow-up				
0-2	25	0.494	0.098	
>2-4	31	0.520	0.087	
>4-6	32	0.497	0.087	
>6-8	31	0.508	0.072	
>8-10	31	0.514	0.090	
>10-12	33	0.532	0.110	
>12-14	23	0.487	0.084	
>14-16	37	0.522	0.085	
>16-18	30	0.559	0.076	
>18	21	0.530	0.087	

[a]Mean ± standard deviation (SD) selenium levels in subcohort members were 0.549 ± 0.125 µg/g for men (*N*=1,061) and 0.575 ± 0.109 µg/g for women (*N*=1,103).

[b]*T*-test of mean toenail selenium level (natural logarithm [ln]-transformed) in first two years of follow-up vs. rest of follow-up years.

Table 5.4. Age- and sex- and multivariable-adjusted[a] associations between toenail selenium levels[b] and risk of head-neck cancer overall; Netherlands Cohort Study, 1986–2006

			Subcohort	Head-neck cancer cases						
				Overall		By sex				
						Men		Women		
	Median (µg/g)[c]		Person-time	No. of	RR[e] (95% CI)	No. of	RR (95% CI)	No. of	RR (95% CI)	*P* for
	Men	Women	at risk (yrs)[d]	cases		cases		cases		interaction[f]
All years of follow-up										
Age- and sex-adjusted analyses										
Q1	0.446	0.477	9,016	112	1 (reference)	94	1 (reference)	18	1 (reference)	
Q2	0.510	0.539	9,222	81	0.69 (0.50-0.95)	61	0.60 (0.42-0.87)	20	1.13 (0.59-2.19)	
Q3	0.565	0.591	9,423	57	0.48 (0.34-0.69)	43	0.42 (0.28-0.63)	14	0.78 (0.38-1.61)	
Q4	0.651	0.683	9,468	44	0.36 (0.25-0.53)	29	0.28 (0.18-0.44)	15	0.81 (0.40-1.64)	0.08
P for trend[g]					<0.001		<0.001		0.40	
Continuous, increment in standard units[h]			37,130	294	0.72 (0.65-0.80)	227	0.68 (0.60-0.78)	67	0.86 (0.72-1.02)	0.04
Multivariable-adjusted analyses										
Q1	0.446	0.477	9,016	112	1 (reference)	94	1 (reference)	18	1 (reference)	
Q2	0.510	0.539	9,222	81	0.81 (0.58-1.14)	61	0.75 (0.50-1.11)	20	1.13 (0.58-2.22)	
Q3	0.565	0.591	9,423	57	0.56 (0.37-0.85)	43	0.50 (0.30-0.82)	14	0.89 (0.42-1.84)	
Q4	0.651	0.683	9,468	44	0.55 (0.37-0.82)	29	0.46 (0.28-0.76)	15	0.89 (0.44-1.83)	0.43
P for trend					0.001		<0.001		0.63	
Continuous, increment in standard units			37,130	294	0.82 (0.73-0.91)	227	0.79 (0.68-0.91)	67	0.89 (0.75-1.06)	0.28
First two years of follow-up excluded										
Multivariable-adjusted analyses										
Q1	0.446	0.477	7,950	100	1 (reference)	84	1 (reference)	16	1 (reference)	
Q2	0.510	0.539	8,146	75	0.83 (0.59-1.18)	56	0.75 (0.50-1.14)	19	1.22 (0.60-2.44)	
Q3	0.565	0.591	8,347	52	0.56 (0.37-0.87)	39	0.49 (0.29-0.82)	13	0.92 (0.43-1.99)	
Q4	0.651	0.683	8,392	42	0.57 (0.38-0.87)	28	0.49 (0.29-0.81)	14	0.93 (0.44-1.96)	0.42
P for trend					0.002		0.001		0.69	
Continuous, increment in standard units			32,835	269	0.82 (0.73-0.92)	207	0.80 (0.69-0.92)	62	0.89 (0.75-1.07)	0.33

Table 5.4 (footnotes).

[a]Adjusted for age (years); sex; cigarette smoking (status (never/former/current), frequency (number of cigarettes per day; continuous, centered), duration (number of years; continuous, centered)); and alcohol consumption (grams ethanol per day; continuous).

[b]Quartiles of toenail selenium (μg/g).

[c]Quartile boundaries (μg/g) men: Q1: 0.183–0.483; Q2: 0.483–0.539; Q3: 0.539–0.601; Q4: 0.602–2.605; women: Q1: 0.109–0.513; Q2: 0.513–0.563; Q3: 0.563–0.622; Q4: 0.622–1.329.

[d]The total person-time at risk was 17,135 person-years for men and 19,995 person-years for women; in sensitivity analyses, men and women had 15,036 and 17,799 person-years at risk, respectively.

[e]Abbreviations: RR: incidence rate ratio; CI: confidence interval; Q: quartile.

[f]*P* value for interaction between sex and toenail selenium level (categorical and continuous), based on cross-product terms in the Cox proportional hazards model and Wald test.

[g]Tests for dose-response trends were assessed by fitting ordinal exposure variables as continuous terms in the Cox proportional hazards model.

[h]For continuous analyses, selenium levels were standardized to the average size of the two central quartiles; one standardized selenium unit equals 0.08 μg/g.

Table 5.5. Age- and sex- and multivariable-adjusted[a] associations between toenail selenium levels[b] and risk of head-neck cancer subtypes; Netherlands Cohort Study, 1986–2006

			Subcohort	Head-neck cancer cases					
				OCC[c]		OHPC[c]		LC[c]	
	Median (μg/g)[d]		Person-time	No. of	RR[e] (95% CI)	No. of	RR (95% CI)	No. of	RR (95% CI)
	Men	Women	at risk (yrs)	cases		cases		cases	
Age- and sex-adjusted analyses									
T1	0.459	0.490	12,149	39	1 (reference)	32	1 (reference)	62	1 (reference)
T2	0.539	0.563	12,394	28	0.69 (0.42-1.13)	17	0.51 (0.28-0.92)	43	0.66 (0.43-0.99)
T3	0.631	0.658	12,587	28	0.68 (0.41-1.12)	13	0.39 (0.20-0.74)	30	0.46 (0.29-0.72)
P for trend[f]					0.14		0.005		0.001
Continuous, increment in standard units[g]			37,130	95	0.82 (0.70-0.96)[i]	62	0.64 (0.52-0.78)	135	0.71 (0.61-0.82)
Men			17,135	54	0.75 (0.59-0.97)	46	0.60 (0.48-0.75)	126	0.69 (0.60-0.81)
Women			19,995	41	0.90 (0.74-1.08)	16	0.74 (0.48-1.15)	9	0.90 (0.62-1.32)
P for interaction[h]					0.28		0.39		0.20
Multivariable-adjusted analyses									
T1	0.459	0.490	12,149	39	1 (reference)	32	1 (reference)	62	1 (reference)
T2	0.539	0.563	12,394	28	0.76 (0.45-1.30)	17	0.59 (0.30-1.17)	43	0.84 (0.54-1.32)
T3	0.631	0.658	12,587	28	0.87 (0.52-1.46)	13	0.59 (0.30-1.14)	30	0.74 (0.45-1.21)[j]
P for trend					0.59		0.10		0.23
Continuous, increment in standard units			37,130	95	0.88 (0.75-1.04)	62	0.72 (0.58-0.90)	135	0.83 (0.71-0.96)
Men			17,135	54	0.85 (0.65-1.12)	46	0.68 (0.52-0.89)	126	0.82 (0.69-0.96)
Women			19,995	41	0.91 (0.75-1.10)	16	0.80 (0.53-1.21)	9	0.95 (0.65-1.38)
P for interaction					0.71		0.51		0.47

[a]Adjusted for age (years); sex; cigarette smoking (status (never/former/current), frequency (number of cigarettes per day; continuous, centered), duration (number of years; continuous, centered)); and alcohol consumption (grams ethanol per day; continuous).

[b]Tertiles of toenail selenium (μg/g).

[c]OCC: oral cavity cancer; OHPC: oro-/hypopharyngeal cancer; LC: laryngeal cancer.

[d]Tertile boundaries (μg/g) men: T1: 0.183–0.502; T2: 0.502–0.575; T3: 0.575–2.605; women: T1: 0.109–0.531; T2: 0.531–0.600; T3: 0.600–1.329.

[e]Abbreviations: RR: incidence rate ratio; CI: confidence interval; T: tertile.

[f]Tests for dose-response trends were assessed by fitting ordinal exposure variables as continuous terms in the Cox proportional hazards model.

[g]For continuous analyses, selenium levels were standardized to the average size of the two central quartiles; one standardized selenium unit equals 0.08 μg/g.

[h]*P* value for interaction between sex and toenail selenium level (continuous), based on cross-product terms in the Cox proportional hazards model and Wald test.

[i]The proportional hazards assumption was possibly violated for the exposure variable in this analysis; there was a statistically significant interaction between the exposure variable and time.

[j]The proportional hazards assumption was possibly violated for the exposure variable in this analysis; there was no statistically significant interaction between the exposure variable and time.

Table 5.6. Multivariable-adjusted[a] associations between toenail selenium (quartiles and standardized units) and risk of head-neck cancer overall, stratified by cigarette smoking status and alcohol consumption; Netherlands Cohort Study, 1986–2006

	Head-neck cancer overall					
	Toenail selenium					
	Q1[bc]	Q2	Q3	Q4	*P* for trend	Standardized unit[d]
Cigarette smoking						
Never						
Cases/person-time at risk (years)	12/3,378	12/3,328	9/3,929	11/4,091		44/14,727
RR (95% CI)	1 (ref)	1.23 (0.50-3.02)	0.75 (0.29-1.95)	0.96 (0.38-2.44)	0.74	0.90 (0.74-1.09)
Former						
Cases/person-time at risk (years)	21/2,431	28/3,337	19/3,569	22/4,111		90/13,447
RR (95% CI)	1 (ref)	0.82 (0.43-1.56)	0.58 (0.29-1.17)	0.60 (0.31-1.16)	0.10	0.86 (0.72-1.04)
Current						
Cases/person-time at risk (years)	79/3,207	41/2,556	29/1,926	11/1,267		160/8,956
RR (95% CI)	1 (ref)	0.76 (0.48-1.20)	0.56 (0.31-1.02)	0.43 (0.21-0.89)	0.007	0.75 (0.64-0.90)
P for interaction[e]	0.94					0.36
Alcohol consumption						
Abstainers						
Cases/person-time at risk (years)	8/2,318	6/2,054	9/2,230	5/2,191		28/8,792
RR (95% CI)	1 (ref)	0.84 (0.29-2.42)	1.36 (0.47-3.95)	0.80 (0.24-2.65)	0.93	0.97 (0.80-1.17)
>0-15 (g/day)						
Cases/person-time at risk (years)	39/4,479	33/4,721	23/5,104	14/5,163		109/19,468
RR (95% CI)	1 (ref)	0.90 (0.53-1.54)	0.58 (0.32-1.03)	0.41 (0.22-0.80)	0.003	0.81 (0.70-0.93)
≥15 (g/day)						
Cases/person-time at risk (years)	65/2,219	42/2,447	25/2,089	25/2,115		157/8,870
RR (95% CI)	1 (ref)	0.65 (0.40-1.05)	0.53 (0.30-0.93)	0.60 (0.34-1.06)	0.04	0.81 (0.67-0.97)
P for interaction	0.32					0.27

[a]Mutually adjusted for age (years), sex, cigarette smoking (status (never/former/current), frequency (number of cigarettes per day; continuous, centered), duration (number of years; continuous, centered)), and alcohol consumption (grams ethanol per day; continuous).

[b]Abbreviations: Q: quartile; RR: incidence rate ratio; CI: confidence interval.

[c]Quartile boundaries (μg/g) men: Q1: 0.183–0.483; Q2: 0.483–0.539; Q3: 0.539–0.601; Q4: 0.602–2.605; women: Q1: 0.109–0.513; Q2: 0.513–0.563; Q3: 0.563–0.622; Q4: 0.622–1.329.

[d]For continuous analyses, selenium levels were standardized to the average size of the two central quartiles; one standardized selenium unit equals 0.08 μg/g.

[e]*P* value for interaction based on cross-product terms in the Cox proportional hazards model and Wald test.

Discussion

In this large prospective cohort study, we found a strong inverse association between toenail selenium status and risk of HNC overall. The association between toenail selenium and risk of HNC overall was considerably stronger among men than women, with a non-significant association in women. Among HNC subtypes, toenail selenium status showed the strongest inverse association with OHPC and LC. Although there was no clear effect modification by cigarette smoking or alcohol consumption in our study, the association we found was most explicit among current smokers. Mechanisms of action of the possible HNC risk reducing effect of selenium probably involve the antioxidant and other anticarcinogenic properties of selenium, including cell cycle regulation.[1,2]

Toenail selenium and risk of HNC and HNC subtypes

Previous observational studies showed mixed results regarding selenium and HNC risk, but most had small numbers. In a population-based case-control study with 379 oral cancer cases in the United States, low toenail selenium status was associated with an increased risk of oral cancer[7] as well, with an odds ratio for low selenium levels in nail tissue (lowest quartile compared with the highest quartile) of 1.4 (95% CI 1.0-2.2). In addition, an inverse association between selenium levels and oral cancer was found in men, but not in women. In a nested case-control study with 28 cases of oral and pharyngeal cancer in the United States[9], smoking-adjusted relative odds for oral cancer were 1.00, 4.26, and 5.43 with increasing tertile levels of serum selenium, in contrast to the inverse association we found. This finding, opposite to ours, may be the result of the very small number of cases in this study by Zheng et al.[9] Furthermore, a nested-case control study in Finland with only 31 HNC cases found a crude relative risk increase per one standard deviation of serum selenium of 1.60 (*P* trend=0.19) for cancer of the lip, oral cavity, and pharynx, and 0.68 (*P* trend=0.39) for laryngeal cancer.[8] A systematic literature review by the World Cancer Research Fund[5] concluded that data regarding selenium and HNC risk were either of too low quality, too inconsistent, or the number of studies too few to allow conclusions to be drawn. Therefore, our large prospective cohort study adds significantly to the existing evidence regarding the association between selenium and HNC risk, strongly suggesting an inverse association.

The differential risk we found between toenail selenium and HNC in men and women was found previously, but in a study of oral cancer.[7] The difference we observed does not seem to be the result of differential smoking behavior among men

and women, as this difference remains after extensive adjustment for smoking. However, toenail selenium status was higher in women than men, which may have accounted for some of the difference, as well as the small number of female cases. It is unclear what other reasons there might be behind this possible difference.

Possible interaction between alcohol, smoking, and toenail selenium

In stratified analyses, we showed that toenail selenium was inversely associated with HNC risk especially in current smokers, with no statistically significant interaction. However, the analysis included only 44 cases among never smokers, and there may be a lack of power to detect a significant deviation from the multiplicative interaction model. In addition, among HNC subtypes, strongest associations were found for OHPC and LC, which we have previously shown to be the subtypes most associated with cigarette smoking.[23] Both observations might point to the possibility that, despite elaborate adjustment for confounding by smoking, there might still be residual confounding. The fact that most associations were substantially stronger in age- and sex-adjusted analyses than in multivariable-adjusted analyses further implies this possibility. On the other hand, it may also indicate that the effect of selenium on HNC risk is associated with smoking, which might be biologically plausible since selenium is an antioxidant and counteracts the oxidative stress caused by smoking.[28] With regard to alcohol consumption, we did not find a statistically significant interaction either, but we cannot rule out possible residual confounding or effect modification.

Strengths and limitations

Strengths of our study are the prospective nature, the completeness and duration of follow-up, and our large case-number. We also had the ability to study HNC subtypes and to adjust for confounders extensively. Finally, we were able to accurately measure selenium in toenails, which reflects long-term selenium intake, and to study a wide range of selenium, including low selenium levels.[2]

A possible limitation of our study is that we only measured selenium at baseline without repeated exposure measurements, which may have led to bias due to misclassification of exposure. In addition, residual confounding due to cigarette smoking and alcohol consumption might have introduced bias, as described above. Furthermore, we have no data on HPV infection. Finally, the higher selenium status in women may have limited the possibility of detecting a significant association in women.

Although it would surely be interesting from a clinical point of view, we believe more research is warranted before putting ours and other findings regarding selenium and HNC risk into lifestyle recommendations or any clinical advice. It would not be

scientifically grounded to make a statement about the exact role of selenium in HNC risk or give any advice based on our results and other available evidence, and we believe we should be conservative doing so for several reasons. Most importantly, it is necessary to examine our results in other large prospective cohort studies first. In addition, numerous other (lifestyle) factors are involved in HNC risk as well, including cigarette smoking and alcohol consumption. Finally, we believe more clinically oriented studies are needed as well before possibly translating our findings into recommendations.

Conclusions

In conclusion, we found an inverse association between toenail selenium level and HNC risk in this large cohort study. Among HNC subtypes, this association was strongest for OHPC and LC. Furthermore, associations of toenail selenium status with HNC risk may be modified by gender, cigarette smoking, and alcohol consumption. Future studies are warranted for confirmation of the association between toenail selenium and HNC risk.

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Chapter 6

Body mass index and risk of subtypes of head-neck cancer: the Netherlands Cohort Study

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Abstract

Background

Low body mass index (BMI) has been associated with risk of head-neck cancer (HNC), but prospective data are scarce. We investigated the association between BMI, BMI at age 20 years, and change in BMI during adulthood with risk of HNC and HNC subtypes.

Methods

120,852 participants completed a questionnaire on diet and other cancer risk factors, including anthropometric measurements, at baseline in 1986. After 20.3 years of follow-up, 411 HNC (127 oral cavity cancer (OCC), 84 oro-/hypopharyngeal cancer (OHPC), and 197 laryngeal cancer (LC)) cases and 3,980 subcohort members were available for case-cohort analysis using Cox proportional hazards models.

Results

BMI at baseline was inversely associated with risk of HNC overall, with a multivariable-adjusted incidence rate ratio of 3.31 (95% CI 1.40-7.82) for subjects with a BMI <18.5 kg/m^2, compared to participants with a BMI of 18.5 to 25 kg/m^2. Among HNC subtypes, this association was strongest for OCC and OHPC. The association between BMI at age 20 and HNC risk appeared to be positive.

Conclusions

In this large prospective cohort study, we found an inverse association between BMI at baseline and HNC risk. For BMI at age 20, however, a positive rather than inverse association was found.

Introduction

Worldwide and in Europe, head and neck cancer (HNC) is the seventh most common type of cancer, including malignancies in the oral cavity, pharynx, and larynx.[1,2] Established risk factors for HNC are cigarette smoking, alcohol consumption and human papillomavirus (HPV) infection.[3,4] A low body mass index (BMI) has also been associated with HNC risk, but this association remains to be clarified. In 2007, the World Cancer Research Fund (WCRF) concluded that data regarding the association between body fatness and HNC risk were insufficient to allow any conclusions to be drawn.[3] Several case-control studies investigated the association between BMI and HNC and mostly found inverse associations. However, since case-control studies are prone to bias, it remains unclear whether the results represent a true inverse association between BMI and HNC or an association due to reverse causality, confounding or effect modification.[3,5-7] Recently, three prospective cohort studies investigated the association between BMI and HNC risk. In the Prostate, Lung, Colorectal, and Ovarian (PLCO) cohort[8], BMI at different time points in life was not associated with HNC risk. The Cancer Prevention Study-II (CPS-II) cohort showed no association between BMI and HNC incidence either, although BMI was inversely associated with HNC mortality in smokers.[9] The National Institutes of Health-AARP (NIH-AARP) Diet and Health Study[10] found that HNC risk was inversely associated with leanness among current smokers, and concluded that the association between leanness and HNC risk may be due to effect modification by smoking.

Given the current evidence, it remains critical to study the association between BMI and HNC risk in prospective cohort studies with comprehensive adjustment for smoking. We therefore examined the association with BMI for HNC and the most frequent HNC subtypes[11]—i.e., oral cavity cancer (OCC), oro-/hypopharyngeal cancer (OHPC), and laryngeal cancer (LC)—within the large prospective Netherlands Cohort Study (NLCS). In addition to BMI at study baseline, we also studied the effects of BMI at age 20 years and change in BMI during adulthood on HNC risk. Finally, we investigated the association of BMI with HNC risk according to smoking status and alcohol consumption.

Methods

Study design and population

The NLCS was initiated in September 1986 and includes 120,852 participants, aged 55-69 years at baseline.[12] The NLCS has been approved by the institutional review board of the TNO Quality of Life Research Institute (Zeist, The Netherlands) and the Medical Ethics Committee of Maastricht University (Maastricht, The Netherlands). All methods were carried out in accordance with the approved guidelines. All cohort members consented to participate in the study by completing and returning the self-administered questionnaire.

We used the case-cohort design for efficiency in data processing and follow-up.[13] Cases were identified from the entire cohort, whereas the number of person-years at risk for the entire cohort was estimated using a subcohort of 5,000 people who were randomly sampled from the total cohort at baseline. Follow-up for cancer incidence was done by record linkage to the Netherlands Cancer Registry (NCR) and the nationwide network and pathology registry (PALGA).[14] Follow-up for vital status of the subcohort was nearly 100% complete after 20.3 years and the completeness of cancer follow-up is estimated to be ≥96%.[15]

We excluded cohort members with prevalent cancer other than skin cancer at baseline (Figure 6.1). Participants with incomplete/inconsistent dietary data or missing data on confounding variables (see below) were also excluded from analysis.[16,17] Only microscopically confirmed first occurrences of squamous cell carcinomas were included.[1,3] These comprise nearly all malignancies of the mouth, pharynx, and larynx.

Data for statistical analysis were available for 3,980 subcohort members and 411 incident cases of the selected HNC subtypes (Figure 6.1). HNC subtypes were classified as proposed by Hashibe et al.[18], according to the International Classification of Diseases for Oncology (ICD-O-3)[19] (Table 6.1). Of the 411 HNC cases, 127 were oral cavity cancer (OCC), 84 were oro-/hypopharyngeal cancer (OHPC), three were oral cavity/pharynx unspecified or overlapping (only included in analyses of HNC overall), and 197 were laryngeal cancer (LC) cases.

Table 6.1. Subclassification of subtypes of head-neck cancer (HNC) as proposed by Hashibe et al.[18], according to the International Classification of Diseases for Oncology, version 3 (ICD-O-3)[19].

HNC-subtype	ICD-O-3
Oral cavity cancer (OCC)	C003-009, C020-C023, C030-C031, C039-C041,C048-C050, C060-C062, C068-C069
Oro-/hypopharyngeal cancer (OHPC)	C019, C024, C051-C052, C090-C091, C098-C104,C108-C109, C129-C132, C138-C139
Oral cavity, pharynx unspecified or overlapping cancer (USC)	C028-C029, C058-C059, C140-C142, C148
Laryngeal cancer (LC)	C320-C329

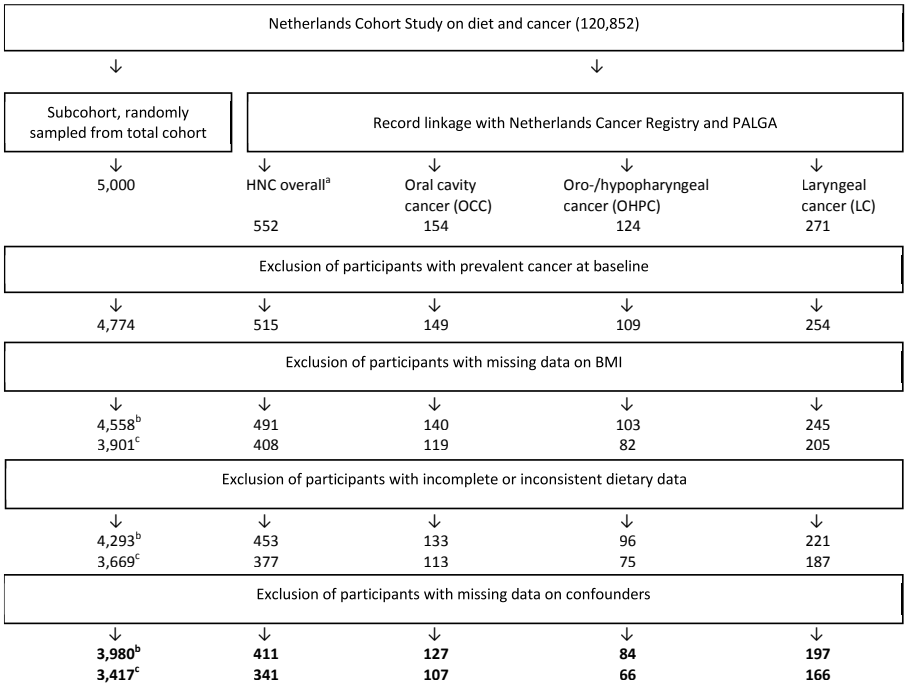

Figure 6.1. Flow diagram of the number of subcohort members and cases on whom the analyses were based.
Abbreviation PALGA: nationwide network and registry of histopathology and cytopathology in the Netherlands.
[a]Oral cavity cancer; oro-/hypopharyngeal cancer; oral cavity, pharynx unspecified or overlapping cancer; laryngeal cancer.
[b]The number of subcohort members or cases used in analyses of BMI at baseline.
[c]The numbers of subcohort members or cases used in analyses of BMI at age 20 and change in BMI.

Questionnaire data

At baseline, all participants completed a self-administered questionnaire about habitual dietary intake, lifestyle habits, and other cancer risk factors, including weight, height, and weight at age 20 years. We asked detailed questions about alcohol consumption and cigarette smoking, as described previously.[20] 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.

BMI at baseline and BMI at age 20 years were calculated using weight at baseline and weight at 20 years, respectively, divided by height at baseline squared (kg/m^2). We classified BMI at baseline according to the World Health Organization (WHO) standard categories: <18.5 (underweight), 18.5 to <25 (normal weight), 25 to <30 (overweight), and ≥30 kg/m^2 (obese). For BMI at age 20 years, categories were <20.0, 20.0 to <21.5, 21.5 to <23, 23 to <25, and ≥25 kg/m^2. We did not use WHO categories here because of few obese cases at the age of 20 years; this classification has been used before in other NLCS analyses.[21] Change in BMI since age 20 years was calculated as BMI at

baseline minus BMI at age 20 years and was classified as <0, 0 to <4, 4 to <8, and ≥8 kg/m^2.[21] Participants with missing values for BMI at baseline were excluded from all analyses; subjects with missing values for BMI at age 20 years were excluded from the analyses of BMI at age 20 years and change in BMI (Figure 6.1).

Statistical analysis

The Cox proportional hazards model was used to estimate age- and sex-adjusted and multivariable-adjusted incidence rate ratios (RR) and corresponding 95% confidence intervals (CI). Person-years at risk were calculated from baseline until diagnosis of HNC, death, emigration, loss to follow-up, or end of follow-up, whichever occurred first. We analyzed BMI at baseline, BMI at age 20 years and change in BMI since age 20 years as described above. For continuous analyses, we used 1 kg/m^2 as increment in BMI.

To evaluate possible reverse causality, we categorized cases according to the year of follow-up in which they were diagnosed and evaluated BMI of HNC cases during the follow-up period. In addition, we used an independent samples t-test to test for statistical significance of differences between the mean BMI of HNC cases diagnosed during the first two years and cases diagnosed later in follow-up. Based on these results (Table 6.2), we decided to include the total follow-up time in our analyses. We also performed sensitivity analyses regarding the association between BMI at baseline and risk of HNC overall by excluding the first two years of follow-up.

The predefined confounders were age (years); sex; alcohol consumption (grams/day); and cigarette smoking (status (never/former/current), number of cigarettes smoked daily, and number of smoking years). We considered the following potential confounders: level of education; non-occupational physical activity; energy-intake; consumption of total vegetables, total fruits, fish, red meat, and meat products (all grams/day); and family history of HNC.[3,22] None of these variables changed the RR for BMI (continuous) for HNC overall or any of the HNC subtypes by >10% when including them in the model. Therefore, the final model included only the predefined confounders. Analyses of change in BMI were also adjusted for BMI at age 20 years. When adjusting for cigarette smoking frequency and duration, we centered these continuous variables as proposed by Leffondré et al.[23]

We assessed tests for linear dose-response trends by fitting ordinal exposure variables as continuous terms. Standard errors were estimated using the robust Huber-White sandwich estimator to account for additional variance due to sampling from the cohort. The proportional hazards assumption was assessed using the scaled Schoenfeld residuals.[24] If there was an indication for violation of the assumption for a variable, it was further investigated by adding a time-varying covariate for that variable to the

Table 6.2. BMI in head-neck cancer (HNC) cases according to sex and time between baseline and HNC diagnosis; Netherlands Cohort Study, 1986–2006

	Head-neck cancer overall			
		BMI (kg/m^2)		
Cases[a]	**No. of cases**	**Mean**	**SD**	***P* value**
All cases	411	24.8	2.7	
Men	318	25.0	2.5	
Women	93	24.3	3.2	
Year of follow-up				
0-2	33	24.9	2.2	
>2	378	24.8	2.8	0.96[b]
Categories year of follow-up				
0-2	33	24.9	2.2	
>2-4	40	24.8	3.0	
>4-6	49	24.8	3.1	
>6-8	44	24.7	2.4	
>8-10	47	24.6	2.9	
>10-12	39	24.6	3.2	
>12-14	44	24.8	2.9	
>14-16	47	25.1	2.6	
>16-18	37	25.2	2.5	
>18	31	24.9	2.3	

[a]Mean ± standard deviation (SD) of BMI at baseline in subcohort members were 24.9 ± 2.6 kg/m^2 for men (*N*=1,954) and 25.0 ± 3.5 kg/m^2 for women (*N*=2,026).

[b]*T*-test of mean BMI at baseline in first two years of follow-up vs. rest of follow-up years.

model. We performed analyses for HNC and all HNC subtypes using a time-varying covariate for current smoking, as described before.[25]

To determine whether sex, cigarette smoking, or alcohol consumption possibly modify the association of BMI with risk of HNC overall, we estimated RRs in strata of these exposures. Tests for interaction were performed with BMI on a continuous scale and *P* values for interaction were assessed by including cross-product terms in the models and performing a Wald test. We performed analyses in strata of alcohol consumption (abstainers; consuming >0 to 15 grams ethanol/day; consuming ≥15 grams ethanol/day) and cigarette smoking status (never/former/current). Alcohol consumption and cigarette smoking were mutually adjusted in these models.

All reported *P* values were based on two-sided tests and considered statistically significant if <0.05. Analyses were done using the Stata 13.1 statistical software package (StataCorp, College Station, Texas, USA).

Results

Baseline characteristics

The mean BMI at baseline of subcohort members (25.0 kg/m^2) and cases (24.8 kg/m^2) was slightly lower in HNC cases (Table 6.3). There was a minor difference between subcohort members and cases regarding BMI at age 20 years (21.5 and 21.9 kg/m^2, respectively), as well as with respect to change in BMI since age 20 years (plus 3.5 and 3.0 kg/m^2, respectively). Among subcohort members and cases, men generally had a similar mean BMI, whereas female cases had a considerably lower BMI at baseline than both male cases and female subcohort members. Notable characteristics with regard to cigarette smoking and alcohol consumption have been described previously[20].

To examine possible reverse causation, we evaluated BMI at baseline of HNC cases during the follow-up period. As we expected, there was no clear pattern in BMI at baseline among HNC cases diagnosed in the course of 20.3 years of follow-up (Table 6.2). HNC overall cases diagnosed after the second year of follow-up (*N*=378) had a mean BMI at baseline of 24.8 kg/m^2, whereas cases diagnosed during the first two years of follow-up (*N*=33) had a mean BMI of 24.9 kg/m^2, a non-statistically significant difference.

Main analyses

Results from age- and sex-adjusted and multivariable-adjusted analyses showed mostly inverse associations between BMI at baseline and risk of HNC overall and HNC subtypes, although these associations were generally somewhat weaker in multivariable-adjusted analyses (Table 6.4).

BMI at baseline was inversely associated with risk of HNC overall, with a multivariable-adjusted RR of 3.31 (95% confidence interval (CI) 1.40-7.82) for subjects with a BMI <18.5 kg/m^2, whereas participants with a BMI ≥30 kg/m^2 had a RR of 0.48 (95% CI 0.22-1.03), both compared to participants with a BMI of 18.5 to 25 kg/m^2 (Table 6.4). The association between BMI at baseline and risk of HNC overall was comparable for men and women and no statistically significant interaction with sex was found (*P*=0.29) for BMI on a continuous scale. Sensitivity analyses showed essentially similar results after exclusion of the first two years of follow-up. We investigated whether the subgroup with underweight at baseline had specific characteristics with regard to smoking and alcohol consumption, since this might have biased the results, but this group was very heterogeneous with regard to these lifestyle aspects.

Among HNC subtypes, BMI at baseline was in general inversely associated with HNC risk as well, with statistically significant associations in OCC (multivariable-adjusted RR comparing participants with a BMI <18.5 kg/m^2 to those with a BMI of 18.5 to 25: 4.49, 95% CI 1.45-13.93) and OHPC (RR: 4.96, 95% CI 1.34-18.33) but not LC (RR: 1.25, 95% CI 0.15-10.31) (Table 6.4). For LC, however, a statistically significant interaction with sex (*P*=0.01) was found, with a decreased risk of LC per kg/m^2 increase in BMI in women (RR: 0.83, 95% CI 0.71-0.97), but the number of female cases was small (*N*=14). We performed sensitivity analyses with only men (*N*=183) in categories of BMI at baseline because of this interaction, but these results showed the same pattern as the results for men and women combined (data not shown).

In contrast to the association between BMI at baseline and HNC risk, the association between BMI at age 20 and HNC risk appeared to be positive rather than inverse, with statistically significant associations on the continuous scale (Table 6.5). Furthermore, point estimates regarding the association between change in BMI since the age of 20 years and HNC risk mostly indicated an inverse association. In addition, we found an interaction between sex and BMI at age 20 years for HNC overall. RRs regarding BMI at age 20 years appeared slightly stronger in multivariable-adjusted analyses compared with age- and sex-adjusted analyses, whereas associations between change in BMI and HNC risk showed both stronger and weaker RRs in multivariable-adjusted analyses (Table 6.5).

Stratified analyses

No statistically significant interaction was found between BMI at baseline and cigarette smoking (*P* interaction=0.86) for HNC overall, nor for BMI at age 20 or change in BMI and cigarette smoking (Table 6.6). A statistically significant interaction was found for both BMI at baseline and at age 20 and alcohol consumption; stratified analyses showed a consistent pattern of the lowest relative risks of HNC overall for BMI at baseline, BMI at age 20, and change in BMI in non-drinkers.

Table 6.3. Characteristics of cases and subcohort members; Netherlands Cohort Study, 1986–2006

	Subcohort	Head-neck cancer cases			
		Overall	Subtypes		
			OCC[a]	OHPC[a]	LC[a]
Exposure variables and confounders[b]	**(*N*=3,980)[c]**	**(*N*=411)[c]**	**(*N*=127)[c]**	**(*N*=84)[c]**	**(*N*=197)[c]**
BMI at baseline (kg/m^2), total	25.0 (3.1)	24.8 (2.7)	24.9 (3.0)	24.4 (2.6)	25.0 (2.6)
Men	24.9 (2.6)	25.0 (2.5)	24.8 (2.8)	24.8 (2.2)	25.2 (2.5)
Women	25.0 (3.5)	24.3 (3.2)	25.1 (3.2)	23.4 (3.2)	22.9 (2.6)
BMI at age 20 years (kg/m^2)[d]	21.5 (2.6)	21.9 (2.5)	22.0 (2.6)	21.7 (2.5)	22.0 (2.4)
Men	21.7 (2.4)	22.2 (2.4)	22.6 (2.4)	22.3 (2.3)	22.0 (2.4)
Women	21.4 (2.7)	21.1 (2.6)	21.4 (2.7)	20.1 (2.2)	21.2 (2.4)
Change in BMI since age 20 years (kg/m^2)[d]	3.5 (3.3)	3.0 (2.9)	2.9 (2.8)	2.8 (2.9)	3.2 (3.0)
Men	3.3 (2.9)	2.9 (2.9)	2.1 (2.7)	2.6 (2.8)	3.3 (3.0)
Women	3.7 (3.6)	3.2 (2.9)	3.7 (2.8)	3.3 (3.1)	1.6 (2.7)
Height (cm)					
Men	177 (7)	176 (7)	176 (7)	177 (6)	176 (7)
Women	165 (6)	166 (6)	165 (5)	167 (7)	167 (8)
Weight (kg)					
Men	77.8 (9.3)	77.9 (9.4)	77.0 (9.7)	77.5 (8.5)	78.4 (9.6)
Women	68.4 (10.2)	66.7 (10.5)	68.2 (9.6)	66.0 (12.0)	64.3 (10.0)
Age at baseline (years)	61.3 (4.2)	61.7 (4.1)	61.9 (4.2)	61.6 (4.0)	61.5 (4.0)
Sex: men (%)	49.1	77.4	57.5	72.6	92.9
Cigarette smoking status (%)					
Never smoker	36.8	13.4	29.1	8.3	5.6
Former smoker	36.0	29.4	26.0	27.4	33.0
Current smoker	27.3	57.2	44.9	64.3	61.4
Ever cigarette smokers:					
Frequency of cigarette smoking (*N*/day)	15.3 (10.3)	19.6 (10.9)	20.5 (11.8)	21.2 (12.8)	18.5 (9.5)
Duration of cigarette smoking (years)	31.6 (12.2)	38.8 (9.7)	36.5 (10.0)	38.5 (9.8)	39.9 (9.5)
Pack-years of cigarette smoking (*N*)	22.7 (17.7)	34.2 (20.9)	34.7 (23.1)	36.5 (23.2)	33.1 (18.8)
Abstainer from alcohol (%)	23.8	11.0	10.2	14.3	10.2

(continued)	**Subcohort**	**Head-neck cancer cases**			
		Overall	**Subtypes**		
			OCC	**OHPC**	**LC**
Exposure variables and confounders	**(*N*=3,980)**	**(*N*=411)**	**(*N*=127)**	**(*N*=84)**	**(*N*=197)**
Alcohol consumers: ethanol intake (g/day)	13.5 (15.1)	26.5 (25.5)	26.2 (25.8)	36.8 (31.5)	22.5 (21.3)
Level of education (%)					
Primary	27.9	27.7	20.5	22.9	33.9
Lower vocational	21.9	17.7	16.5	18.1	18.5
Secondary and medium vocational	35.8	35.3	40.9	37.4	31.3
University and higher vocational	14.4	19.4	22.1	21.7	16.4
Non-occupational physical activity (min/day)	73 (60)	73 (59)	69 (59)	69 (56)	76 (59)
Energy intake (kJ/day)					
Men	9,084 (2,133)	9,069 (2,132)	8,686 (2,242)	9,100 (1,843)	9,227 (2,163)
Women	7,064 (1,651)	6,805 (1,940)	6,624 (2,055)	7,153 (1,607)	6,992 (1,841)
First-grade family history of HNC (%)	1.8	2.0	1.6	1.2	2.0

[a]OCC: oral cavity cancer; OHPC: oro-/hypopharyngeal cancer; LC: laryngeal cancer.

[b]Values are given as mean (SD); for categorical variables, *N* (%) is presented.

[c]The number of subcohort members or cases (with complete data on BMI at baseline, age, sex, cigarette smoking, and alcohol consumption), used in analyses of BMI at baseline.

[d]The numbers of subcohort members or cases (with complete data on BMI at baseline, BMI at age 20, and change in BMI, age, sex, cigarette smoking, and alcohol consumption), used in analyses of BMI at age 20 and change in BMI: 3,417 subcohort members; 341 HNC overall, 107 OCC, 66 OHPC, and 166 LC cases.

Table 6.4. Age- and sex- and multivariable-adjusted[a] associations between BMI at baseline[b] and risk of head-neck cancer subtypes; Netherlands Cohort Study, 1986–2006

			Subcohort	Head-neck cancer cases							
				Overall		Subtypes					
						OCC[c]		OHPC[c]		LC[c]	
	Median (kg/m^2)		Person-time	No.	RR[d] (95% CI)	No.	RR (95% CI)	No.	RR (95% CI)	No.	RR (95% CI)
	Men	Women	at risk (yrs)	cases		cases		cases		cases	
All years of follow-up											
Age- and sex-adjusted analyses											
<18.5	17.8	17.8	524	8	4.23 (1.85-9.64)	4	5.19 (1.72-15.66)[g]	3	7.22 (2.14-24.32)	1	1.54 (0.19-12.27)
18.5 to <25	23.5	23.1	36,503	214	1 (reference)	64	1 (reference)	44	1 (reference)	103	1 (reference)
25 to <30	26.5	26.8	25,976	178	1.08 (0.87-1.34)	53	1.12 (0.77-1.62)	37[h]	0.99 (0.64-1.54)	88	1.08 (0.80-1.45)
≥30	31.0	32.0	4,249	11	0.60 (0.32-1.14)	6	0.92 (0.39-2.17)			5	0.68 (0.27-1.72)
P for trend[e]					0.15		0.55		0.35		0.80
Continuous (per 1 kg/m^2)			67,251	411	0.98 (0.94-1.02)	127	0.99 (0.93-1.06)	84	0.93 (0.86-1.01)	197	1.01 (0.95-1.06)
Men			31,101	318	1.01 (0.96-1.06)	73	0.98 (0.87-1.09)	61	0.98 (0.89-1.08)	183	1.03 (0.97-1.09)
Women			36,150	93	0.93 (0.87-0.99)	54	1.01 (0.94-1.08)	23	0.85 (0.74-0.99)	14	0.81 (0.69-0.95)
P for interaction[f]					0.06		0.65		0.12		0.005
Multivariable-adjusted analyses											
<18.5	17.8	17.8	524	8	3.31 (1.40-7.82)	4	4.49 (1.45-13.93)[g]	3	4.96 (1.34-18.33)	1	1.25 (0.15-10.31)
18.5 to <25	23.5	23.1	36,503	214	1 (reference)	64	1 (reference)	44	1 (reference)	103	1 (reference)
25 to <30	26.5	26.8	25,976	178	1.11 (0.88-1.40)	53	1.18 (0.80-1.75)	37[h]	1.01 (0.62-1.64)	88	1.06 (0.78-1.45)
≥30	31.0	32.0	4,249	11	0.48 (0.22-1.03)	6	0.69 (0.25-1.88)			5	0.62 (0.23-1.64)
P for trend					0.12		0.41		0.45		0.69
Continuous (per 1 kg/m^2)			67,251	411	0.98 (0.94-1.02)	127	0.98 (0.92-1.05)	84	0.92 (0.85-1.00)[i]	197	1.00 (0.95-1.06)
Men			31,101	318	0.99 (0.94-1.05)	73	0.94 (0.84-1.06)	61	0.94 (0.85-1.04)	183	1.03 (0.97-1.09)
Women			36,150	93	0.95 (0.89-1.01)	54	1.01 (0.95-1.08)	23	0.89 (0.78-1.01)	14	0.83 (0.71-0.97)
P for interaction					0.29		0.31		0.50		0.01
First two years of follow-up excluded											
Multivariable-adjusted analyses											
<18.5	17.8	17.8	452	8	3.87 (1.63-9.22)[g]	4	4.91 (1.57-15.35)[i]	3	4.93 (1.33-18.27)	1	1.72 (0.20-14.64)
18.5 to <25	23.5	23.1	32,255	197	1 (reference)	60	1 (reference)	43	1 (reference)	91	1 (reference)
25 to <30	26.5	26.8	22,907	162	1.11 (0.87-1.41)	50	1.19 (0.80-1.77)	36[h]	1.01 (0.62-1.65)	76	1.04 (0.75-1.45)
≥30	31.0	32.0	3,751	11	0.52 (0.24-1.14)	6	0.76 (0.28-2.04)			5	0.72 (0.27-1.92)

(continued)			Subcohort	Head-neck cancer cases							
				Overall		Subtypes					
						OCC		OHPC		LC	
	Median (kg/m^2)		Person-time	No.	RR (95% CI)	No.	RR (95% CI)	No.	RR (95% CI)	No.	RR (95% CI)
	Men	Women	at risk (yrs)	cases		cases		cases		cases	
P for trend					0.13		0.46		0.46		0.73
Continuous (per 1 kg/m^2)			59,364	378	0.98 (0.94-1.02)	120	0.99 (0.93-1.05)	82	0.92 (0.85-1.00)	173	1.00 (0.94-1.07)
Men			27,244	291	1.00 (0.94-1.06)	68	0.97 (0.86-1.09)	59	0.95 (0.85-1.05)	163	1.03 (0.97-1.10)
Women			32,120	87	0.94 (0.88-1.00)[i]	52	1.00 (0.94-1.08)	23	0.89 (0.78-1.01)	10	0.72 (0.62-0.85)
P for interaction					0.16		0.57		0.47		<0.001

[a]Adjusted for age (years); sex; cigarette smoking (status (never/former/current), frequency (number of cigarettes per day; continuous, centered), duration (number of years; continuous, centered)); and alcohol consumption (grams ethanol per day; continuous).

[b]Categories of BMI (kg/m^2).

[c]OCC: oral cavity cancer; OHPC: oro-/hypopharyngeal cancer; LC: laryngeal cancer.

[d]Abbreviations: RR: incidence rate ratio; CI: confidence interval.

[e]Tests for dose-response trends were assessed by fitting ordinal exposure variables as continuous terms in the Cox proportional hazards model.

[f]*P* value for interaction between sex and BMI at baseline (continuous), based on cross-product terms in the Cox proportional hazards model and Wald test.

[g]The proportional hazards assumption was possibly violated for the exposure variable in this analysis; there was a statistically significant interaction between the exposure variable and time.

[h]For analyses regarding BMI at baseline and OHPC, BMI was categorized into three categories (<18.5; 18.5 to <25; and ≥25 kg/m^2) because there were no OHPC cases with a BMI ≥30 kg/m^2.

[i]*P*<0.05.

[j]The proportional hazards assumption was possibly violated for the exposure variable in this analysis; there was no statistically significant interaction between the exposure variable and time.

Table 6.5. Age- and sex- and multivariable-adjusted[a] associations between BMI at age 20, change in BMI since age 20[b] and risk of head-neck cancer subtypes; Netherlands Cohort Study, 1986–2006

			Subcohort	Head-neck cancer cases							
				Overall		Subtypes					
						OCC[c]		OHPC[c]		LC[c]	
	Median (kg/m^2)		Person-time	No.	RR[d] (95% CI)	No.	RR (95% CI)	No.	RR (95% CI)	No.	RR (95% CI)
	Men	Women	at risk (yrs)	cases		cases		cases		cases	
BMI at age 20											
Age- and sex-adjusted analyses											
<20.0	18.9	18.6	14,979	69	0.88 (0.63-1.23)	24	0.96 (0.54-1.70)	14	0.80 (0.40-1.59)	30	0.85 (0.53-1.38)
20.0 to <21.5	20.8	20.8	14,085	91	1 (reference)	25	1 (reference)	20	1 (reference)	46	1 (reference)
21.5 to <23	22.2	22.1	14,138	73	0.82 (0.59-1.14)	24	0.97 (0.55-1.71)	14	0.72 (0.36-1.43)	34	0.76 (0.48-1.21)
23 to <25	23.9	23.7	10,575	73	1.11 (0.79-1.55)	22	1.20 (0.67-2.16)	12	0.83 (0.40-1.72)	39	1.18 (0.76-1.86)
≥25	26.0	26.0	4,199	35	1.43 (0.93-2.19)	12	1.71 (0.84-3.48)	6	1.11 (0.44-2.85)	17	1.42 (0.78-2.56)
P for trend[e]					0.06		0.16		0.81		0.12
Continuous (per 1 kg/m^2)			57,977	341	1.05 (1.01-1.10)	107	1.08 (1.01-1.15)	66	1.02 (0.93-1.12)	166	1.05 (0.99-1.12)
Men			25,019	258	1.09 (1.03-1.15)	56	1.16 (1.05-1.29)	48	1.11 (1.00-1.24)	153	1.06 (0.99-1.13)
Women			32,958	83	0.96 (0.88-1.05)	51	1.01 (0.91-1.12)	18	0.83 (0.70-0.98)	13	0.98 (0.82-1.18)
P for interaction[f]					0.01		0.05		0.004		0.44
Multivariable-adjusted analyses											
<20.0	18.9	18.6	14,979	69	0.78 (0.54-1.11)	24	0.85 (0.47-1.55)[i]	14	0.63 (0.29-1.34)	30	0.78 (0.47-1.28)
20.0 to <21.5	20.8	20.8	14,085	91	1 (reference)	25	1 (reference)	20	1 (reference)	46	1 (reference)
21.5 to <23	22.2	22.1	14,138	73	0.82 (0.58-1.15)	24	0.97 (0.55-1.73)	14	0.74 (0.36-1.50)	34	0.75 (0.46-1.20)
23 to <25	23.9	23.7	10,575	73	0.94 (0.65-1.35)	22	1.02 (0.55-1.89)	12	0.59 (0.25-1.36)[j]	39	1.05 (0.66-1.66)
≥25	26.0	26.0	4,199	35	1.45 (0.93-2.27)	12	1.75 (0.85-3.61)[i]	6	1.17 (0.45-3.04)	17	1.36 (0.74-2.51)
P for trend					0.07		0.14		0.76		0.15
Continuous (per 1 kg/m^2)			57,977	341	1.06 (1.01-1.11)	107	1.08 (1.01-1.16)	66	1.03 (0.93-1.14)	166	1.05 (0.98-1.12)
Men			25,019	258	1.10 (1.03-1.16)	56	1.17 (1.05-1.30)	48	1.12 (0.99-1.26)	153	1.06 (0.99-1.13)
Women			32,958	83	0.97 (0.89-1.05)	51	1.01 (0.92-1.12)	18	0.85 (0.73-1.00)[h]	13	0.98 (0.82-1.18)
P for interaction					0.01		0.05		0.007		0.47

(continued)	Median (kg/m^2)		Person-time	No.	RR (95% CI)	No.	RR (95% CI)	No.	RR (95% CI)	No.	RR (95% CI)
	Men	Women	at risk (yrs)	cases		cases		cases		cases	
Change in BMI since age 20[g]											
Age- and sex-adjusted analyses											
<0	-1.2	-1.5	6,610	48	1.33 (0.94-1.89)	10	0.73 (0.37-1.46)	11	1.57 (0.78-3.15)	26	1.68 (1.05-2.69)
0 to <4	2.2	2.3	27,953	173	1 (reference)	60	1 (reference)	33	1 (reference)	79	1 (reference)
4 to <8	5.5	5.5	18,489	103	0.94 (0.72-1.22)	32	0.79 (0.51-1.23)	19	0.89 (0.50-1.59)	52	1.09 (0.75-1.58)
≥8	9.2	9.4	4,926	17	0.75 (0.44-1.28)	5	0.51 (0.20-1.26)	3	0.67 (0.20-2.21)	9	1.05 (0.51-2.19)
P for trend					0.05[h]		0.27		0.15		0.32
Continuous (per 1 kg/m^2)			57,977	341	0.95 (0.92-0.99)	107	0.94 (0.89-0.99)	66	0.94 (0.87-1.01)	166	0.98 (0.93-1.04)
Men			25,019	258	0.95 (0.90-1.00)[h]	56	0.86 (0.78-0.94)	48	0.92 (0.83-1.02)	153	1.00 (0.94-1.06)
Women			32,958	83	0.96 (0.92-1.01)	51	1.00 (0.94-1.06)	18	0.97 (0.87-1.08)	13	0.86 (0.79-0.94)
P for interaction					0.65		0.008		0.48		0.007
Multivariable-adjusted analyses											
<0	-1.2	-1.5	6,610	48	1.25 (0.84-1.86)	10	0.65 (0.30-1.38)	11	1.75 (0.78-3.96)	26	1.49 (0.88-2.53)
0 to <4	2.2	2.3	27,953	173	1 (reference)	60	1 (reference)	33	1 (reference)	79	1 (reference)
4 to <8	5.5	5.5	18,489	103	1.00 (0.74-1.34)	32	0.87 (0.55-1.37)[i]	19	0.90 (0.47-1.69)	52	1.16 (0.77-1.74)
≥8	9.2	9.4	4,926	17	0.72 (0.39-1.32)	5	0.51 (0.20-1.33)	3	0.46 (0.10-2.03)[i]	9	1.07 (0.49-2.34)
P for trend					0.19		0.55		0.13		0.71
Continuous (per 1 kg/m^2)			57,977	341	0.96 (0.92-1.01)	107	0.95 (0.89-1.01)	66	0.92 (0.84-1.01)	166	1.00 (0.94-1.07)
Men			25,019	258	0.95 (0.90-1.01)	56	0.86 (0.78-0.96)	48	0.89 (0.79-1.00)[h]	153	1.02 (0.95-1.09)
Women			32,958	83	0.98 (0.93-1.03)	51	1.01 (0.95-1.07)	18	0.97 (0.87-1.09)	13	0.89 (0.81-0.98)
P for interaction					0.43		0.006		0.21		0.01

[a]Adjusted for age (years), sex, cigarette smoking (status (never/former/current), frequency (number of cigarettes per day; continuous, centered), duration (number of years; continuous, centered)), and alcohol consumption (grams ethanol per day; continuous).

[b]Categories of BMI at age 20 and change in BMI (kg/m^2).

[c]OCC: oral cavity cancer; OHPC: oro-/hypopharyngeal cancer; LC: laryngeal cancer.

[d]Abbreviations: RR: incidence rate ratio; CI: confidence interval.

[e]Tests for dose-response trends were assessed by fitting ordinal exposure variables as continuous terms in the Cox proportional hazards model.

[f]*P* value for interaction between sex and BMI at baseline (continuous), based on cross-product terms in the Cox proportional hazards model and Wald test.

[g]Change in BMI since age 20 years was additionally adjusted for BMI at age 20 years.

[h]$P<0.05$.

[i]The proportional hazards assumption was possibly violated for the exposure variable in this analysis; there was no statistically significant interaction between the exposure variable and time.

[j]The proportional hazards assumption was possibly violated for the exposure variable in this analysis; there was a statistically significant interaction between the exposure variable and time.

Table 6.6. Multivariable-adjusted[a] associations between BMI[b] and risk of head-neck cancer overall, stratified by cigarette smoking status and alcohol consumption; Netherlands Cohort Study, 1986–2006

Head-neck cancer overall				
Cigarette smoking	**Never**	**Former**	**Current**	***P* interaction[c]**
BMI at baseline				
Cases/ person-time at risk (years)	55/26,337	121/23,911	235/17,003	0.86
RR[d] (95% CI)	1.00 (0.91-1.09)	0.98 (0.90-1.06)	0.97 (0.92-1.02)	
BMI at age 20				
Cases/ person-time at risk (years)	46/23,259	104/20,440	191/14,278	0.85
RR (95% CI)	1.10 (1.00-1.21)[f]	1.05 (0.96-1.14)	1.06 (0.99-1.13)	
Change in BMI since age 20[e]				
Cases/ person-time at risk (years)	46/23,259	104/20,440	191/14,278	0.94
RR (95% CI)	0.96 (0.87-1.05)	0.97 (0.88-1.07)	0.96 (0.90-1.02)	
Alcohol consumption	**Non-drinkers**	**>0-15 (g/day)**	**≥15 (g/day)**	
BMI at baseline				
Cases/ person-time at risk (years)	45/15,880	156/34,959	210/16,413	0.05[f]
RR (95% CI)	0.86 (0.78-0.95)	1.01 (0.96-1.07)	0.99 (0.93-1.05)	
BMI at age 20				
Cases/ person-time at risk (years)	41/13,675	136/30,240	164/14,062	0.01
RR (95% CI)	0.90 (0.81-1.00)[g]	1.08 (1.00-1.16)[f]	1.08 (1.01-1.16)	
Change in BMI since age 20[e]				
Cases/ person-time at risk (years)	41/13,675	136/30,240	164/14,062	0.77
RR (95% CI)	0.88 (0.79-0.99)	0.99 (0.93-1.06)	0.96 (0.90-1.03)	

[a]Mutually adjusted for age (years); sex; cigarette smoking (status (never/former/current), frequency (number of cigarettes per day; continuous, centered), duration (number of years; continuous, centered)); and alcohol consumption (grams ethanol per day; continuous).

[b]Continuous (per 1 kg/m^2 increment).

[c]*P* value for interaction based on cross-product terms in the Cox proportional hazards model and Wald test.

[d]Abbreviations: RR: incidence rate ratio; CI: confidence interval.

[e]Change in BMI since age 20 years was additionally adjusted for BMI at age 20 years.

[f]$P<0.05$.

[g]$P>0.05$.

Discussion

In this large prospective cohort study, we found an inverse association between BMI at baseline and risk of HNC overall. Among HNC subtypes, BMI at baseline showed the strongest inverse association with OCC and OHPC. For BMI at age 20, on the other hand, we found a positive rather than inverse association, whereas the association between change in BMI since the age of 20 years and HNC risk appeared to be inverse again. Finally, there was effect modification by alcohol consumption in our study, with the lowest risks of HNC overall for BMI at baseline, BMI at age 20, and change in BMI in non-drinkers.

BMI and risk of HNC and HNC subtypes

Previous studies showed mixed results regarding BMI and HNC risk. Case-control studies largely indicated an inverse association between BMI and HNC risk[3], but a systematic literature review by the WCRF[3] concluded that data regarding the association between body fatness and HNC risk—based on case-control studies—were insufficient to allow conclusions to be drawn. Since then, a large pooled analysis[5] of 17 case-control studies with 12,716 cases and 17,438 controls showed that leanness (BMI <18.5 kg/m^2) was associated with increased HNC risk, regardless of smoking and drinking status. Furthermore, three prospective cohort studies examined the association between BMI and HNC risk. The CPS-II cohort[9] included 340 HNC cases and showed no association between BMI and HNC incidence. There was no effect modification by smoking status. In the PLCO cohort[8], with 177 cases, neither BMI at different time points in life nor changes in BMI were associated with HNC risk. Recently, the NIH-AARP Diet and Health Study[10], which comprised 779 cases, showed evidence for an inverse relationship between BMI at baseline and HNC risk, in particular OCC and OHPC, but none of the associations were statistically significant. In addition, BMI at earlier ages showed no association with HNC risk. When stratified by smoking, the inverse association was only observed among current (and not former) smokers (hazard ratio 0.76 per 5 kg/m^2 increase, 95% CI 0.63-0.93); also, the association diminished as initial years of follow-up were excluded. None of the three cohort studies investigated effect modification by alcohol intake.

The results from our prospective cohort study partly confirm findings from previous—both case-control and prospective—studies. As most case-control studies and the NIH-AARP cohort study[10], we also found an inverse association between BMI and HNC risk, and—like NIH-AARP—with strongest associations for OCC and OHPC. The CPS-II[9] and the PLCO[8] cohort, on the other hand, did not find an inverse association

between BMI and HNC risk. Unlike previous cohort studies[8,10], we also found a positive association with regard to BMI at age 20 and HNC risk, and an interaction with alcohol consumption. Finally, we did not find an interaction with smoking status, although this might have to do with a lack of power (see below).

Possible mechanisms regarding BMI and HNC risk

The question remains whether the inverse association we found between BMI at baseline and HNC risk is a true effect by BMI, or an effect based on reverse causality or confounding by smoking, alcohol consumption, or other factors. We cannot clearly explain why we found a positive rather than inverse association between BMI at age 20 and HNC risk, whilst BMI at baseline was in general inversely associated with HNC risk. Given the contrast in our results regarding the associations between BMI at baseline, BMI at age 20, and HNC risk, it appears that leanness itself is probably not a causal factor in this association. The fact that some associations were weaker—but others stronger—in multivariable-adjusted analyses than in age- and sex-adjusted analyses implies the possibility of residual confounding. Reverse causality might play a role in the association between BMI at baseline and HNC risk. However, sensitivity analyses showed similar results for different periods of follow-up, which makes reverse causality unlikely.

Strengths and limitations

Strengths of our study are the prospective nature, our large case-number, and the completeness and duration of follow-up. In addition, we had the ability to study HNC subtypes and to adjust for confounders thoroughly. A possible limitation of our study is that the data on BMI in our study are self-reported, which may have led to bias due to misclassification of exposure. BMI at age 20 years was calculated using self-reported weight at age 20 years and this might have introduced recall bias; however, we expect this to be non-differential. Despite thorough adjustment for confounding by smoking and alcohol consumption, we cannot rule out residual confounding, as described above. Furthermore, in stratified analyses, we did not find a statistically significant interaction with regard to cigarette smoking. However, the analysis included only 55 cases among never smokers, mainly females, and there may have been a lack of power to detect a significant interaction. Finally, we lack data on HPV infection.[20]

Conclusions

In conclusion, we found an inverse association between BMI at baseline and HNC risk in this large cohort study. Among HNC subtypes, this association was strongest for OCC

and OHPC. For BMI at age 20, however, a positive rather than inverse association was found. Furthermore, associations of BMI with HNC risk may be modified by alcohol consumption. We conclude that leanness itself is probably not a causal factor in the association with HNC. Future studies are warranted for further clarifications of the possible mechanisms involved regarding BMI and HNC risk.

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Chapter 7

Discussion

In this chapter, we will discuss the main findings of our study regarding the associations between lifestyle factors and the risk of head-neck cancer (HNC) and HNC subtypes. We will put our findings into perspective with the existing evidence and attempt to create an integrated view regarding the etiology of HNC. Furthermore, we will discuss the strengths and limitations of our study. Finally, recommendations for future research will be given.

Main findings

We studied possible lifestyle factors regarding risk of HNC and HNC subtypes, i.e., oral cavity cancer (OCC), oro-/hypopharyngeal cancer (OHPC), and laryngeal cancer (LC), within the large prospective Netherlands Cohort Study (NLCS). Our study is one of the largest prospective cohort studies on lifestyle factors and HNC risk, in particular HNC subtypes, and our findings—summarized in Table 7.1—therefore add significantly to the existing literature. We will further elaborate on this in the next paragraph.

First, we investigated the association between alcohol consumption, cigarette smoking and risk of HNC (subtypes). We found that both alcohol consumption and cigarette smoking were strongly, independently associated with an increased risk of HNC (**Chapter 2**). In addition, we found a positive, multiplicative interaction between both factors with regard to HNC risk. The strength of these associations, however, differed among HNC subtypes; OCC was most strongly associated with alcohol consumption but most weakly with cigarette smoking, whereas LC was not statistically significantly associated with alcohol consumption.

Next, we found that total vegetable and fruit consumption was inversely associated with risk of HNC overall and all HNC subtypes (**Chapter 3**), with the strongest inverse associations for OCC. Total vegetable consumption was inversely associated with risk of HNC overall as well; associations with HNC subtypes were also inverse and were largely similar for OCC, OHPC, and LC. Total fruit consumption was associated with reduced risks of HNC overall and all subtypes; the strongest associations were with OCC. Associations between vegetable and fruit consumption and HNC risk were not clearly modified by cigarette smoking status or alcohol consumption.

In **Chapter 4**, we examined associations of vitamin and carotenoid intake with HNC risk. Strong inverse associations were found between vitamin C and HNC overall, OCC and OHPC. No clear associations were found for other nutrients and vitamin supplementation; however, most point estimates showed possible protective effects.

Table 7.1. Main findings of the association between lifestyle factors and risk of head-neck cancer subtypes; Netherlands Cohort Study, 1986–2006

		Direction and strength of observed association				
Risk factor	Exposure	HNC overall	OCC	OHPC	LC	Chapter
Alcohol consumption	≥30 grams ethanol per day vs. abstainers	++	++	++	(+)	2
Cigarette smoking	Current vs. never smokers	++	++	++	++	2
Vegetable and fruit consumption	Quartiles; Q4 vs. Q1	-	--	-	0	3
Vegetable consumption	Quartiles; Q4 vs. Q1	-	(-)	(-)	0	3
Fruit consumption	Quartiles; Q4 vs. Q1	(-)	-	(-)	0	3
Vitamin C intake	Quartiles; Q4 vs. Q1	--	--	--	(-)	4
Vitamin C supplement	Supplement use yes vs. no	0	0	(+)	0	4
Vitamin E intake	Quartiles; Q4 vs. Q1	0	0	(+)	0	4
Vitamin E supplement	Supplement use yes vs. no	0	0	0	0	4
Carotenoids (in general)	Quartiles; Q4 vs. Q1	0	?	?	0	4
Toenail selenium status	Quartiles (Q4 vs. Q1): HNC overall; tertiles (T3 vs. T1): HNC subtypes	-	0	(-)	(-)	5
Leanness (BMI <18.5) at baseline	BMI <18.5 kg/m^2 vs. normal weight (18.5-25 kg/m^2)	++[a]	++	++	0	6
Overweight (BMI 25-30) at baseline	BMI 25-30 kg/m^2 vs. normal weight (18.5-25 kg/m^2)	0	0	0	0	6
Obesity (BMI ≥30) at baseline	BMI ≥30 kg/m^2 vs. normal weight (18.5-25 kg/m^2)	(--)[b]	(-)	[c]	(-)	6
Leanness (BMI <20) at age 20[d]	BMI <20 kg/m^2 vs. 20-21.5 kg/m^2	(-)	0	(-)	(-)	6
Overweight and obese[d] (BMI ≥25) at age 20	BMI ≥25 kg/m^2 vs. 20-21.5 kg/m^2	(+)	(+)	0	(+)	6

++,--: strong positive or inverse association (incidence rate ratio (RR) ≥2.0 or RRs <0.5); statistically significant, either RR or *P* for trend.

(++),(--): strong positive or inverse association (RRs ≥2.0 or RRs <0.5); not statistically significant.

+,-: positive (RRs <2.0–>1.25) or inverse (RRs >0.5–<0.8) association; statistically significant.

(+),(-): positive (RRs <2.0–>1.25) or inverse (RRs >0.5–<0.8) association; not statistically significant.

0: no association (RRs very close to 1 (≥0.8–≤1.25) and no dose-response trends).

?: inconsistent association.

[a]Based on only 8 cases.

[b]Based on only 11 cases.

[c]There were no OHPC cases with a BMI ≥30 kg/m^2.

[d]For BMI at age 20, we used different categories (no category obesity) because of few cases of BMI <18.5 (*N*=7) and BMI ≥30 (*N*=10) at the age of 20 years.

Furthermore, the association between vitamin E intake and the risk on HNC overall was modified by alcohol consumption.

We found an inverse association between toenail selenium level and HNC risk (**Chapter 5**). Among HNC subtypes, this association was strongest for OHPC and LC. The association between toenail selenium and risk of HNC overall was considerably stronger among men than women. Although there was no clear effect modification by cigarette smoking or alcohol consumption in our study, the association we found was most explicit among current smokers. Associations of toenail selenium status with HNC risk may be modified by gender, cigarette smoking, and alcohol consumption.

Finally, we found an inverse association between BMI at baseline and HNC risk (**Chapter 6**). Among HNC subtypes, this association was strongest for OCC and OHPC. For BMI at age 20, on the other hand, we found a positive rather than inverse association, whereas the association between change in BMI since the age of 20 years and HNC risk appeared to be inverse again. There was effect modification by alcohol consumption in our study, with the lowest risks of HNC overall for BMI at baseline, BMI at age 20, and change in BMI in non-drinkers.

Interpretation of study findings

When putting the lifestyle factors we investigated in perspective with regard to HNC risk, what can we say? First, our study confirms that alcohol consumption and cigarette smoking are main risk factors for HNC.[1] They strongly increase HNC risk, and when used both, the risk is even higher. Alcoholic beverages and acetaldehyde, the main metabolite of ethanol, are classified as a class I carcinogen[2], and it is plausible that alcohol acts both directly and indirectly—after being metabolized—in HNC carcinogenesis.[3,4] Cigarette smoking contains numerous carcinogenic substances, e.g., nicotine and benzene, which largely explain the increased HNC risk.[5] In addition, tobacco carcinogens can solve in alcohol, which may explain the interaction between smoking and alcohol consumption.[3,4] The differential risk among HNC subtypes may be explained by the larynx having the least direct exposure to alcohol—yet the most to cigarette smoke—compared with the oral cavity and pharynx (next paragraph).[4,6]

Furthermore, we clearly showed that other lifestyle factors significantly influence HNC risk as well. Vegetable and fruit consumption, as well as vitamin C intake, was associated with a significantly decreased HNC risk. The intake of a vitamin C supplement, vitamin E, and carotenoids, however, did not clearly show an association with the risk of developing HNC in our study. Finally, a low toenail selenium status was

related to an increased HNC risk, as well as being underweight (BMI <18.5 kg/m^2) at baseline.

We can thus conclude that nutrition and lifestyle factors other than smoking and alcohol consumption can clearly influence HNC risk. But in which ways does this effect occur? When we look at the big picture of our results, the anticarcinogenic and antioxidant properties of vegetables, fruits, vitamin C and selenium probably play an important role.[3,7,8] The potential anticarcinogenic properties of numerous components present in vegetables and fruits include vitamins, fiber, folate, flavonoids and carotenoids, some of which are potential antioxidants and might play a role in several processes, such as protection against oxidative stress, normal cell proliferation and differentiation, and DNA repair.[3,7] Selenium, an essential trace element present in food, probably influences carcinogenesis by its antioxidant properties.[8]

Antioxidants likely counteract oxidative stress, which is amongst others caused by smoking.[3] However, we showed no clear interaction of the lifestyle factors we studied with smoking (or alcohol); had there been an interaction, we would have expected to see a protective effect of, e.g., vegetables and fruits especially among smokers. This might suggest that nutrition is an important part of a healthy lifestyle and diminishes HNC risk, whether someone is a cigarette smoker and/or alcohol consumer or not. Still, our results might be influenced by power issues, mainly because of the low number of (male) never smokers in our cohort, and epidemiological evidence about potential interaction effects on HNC risk remains therefore inconclusive.

Few cohort studies have investigated lifestyle factors and HNC risk.[9-13] The National Institutes of Health-AARP (NIH-AARP) Diet and Health cohort[13-15], with 490,802 United States participants and 787 cases, supports the hypotheses that alcohol consumption and cigarette smoking increase HNC risk, and that total vegetable and fruit intake is associated with reduced HNC risk. However, this large study lacks information on smoking duration, a relevant aspect of smoking behavior. In the prospective European Investigation Into Cancer and Nutrition (EPIC) study[9,16] with 345,904 subjects and 352 cases (164 oral cavity and pharynx, 84 esophagus, and 104 larynx), intake of fruits and vegetables was also linked with reduced risk of upper aero-digestive tract cancer (UADTC), whereas alcohol increased UADTC risk. Yet, HNC was combined with esophageal cancer in EPIC, which may lead to other overall associations. Chyou et al.[10] examined diet, alcohol, smoking and UADTC risk in a prospective cohort study among 7,995 Japanese-American men, but with only 92 cases. Again, cigarette smoking and alcohol consumption increased UADTC risk, whereas consumption of fruit was also inversely associated with risk of UADTC. A prospective study of Norwegian men by Kjaerheim et al.[12] investigated the role of alcohol, tobacco, and dietary factors

in upper aerogastric tract cancers (UAGT) and had similar findings regarding alcohol, smoking, fruit intake and risk of UAGT. However, the study only included 71 cases (28 oral and pharyngeal, 21 laryngeal, and 22 esophageal cancer cases), analyzed altogether. Lastly, the prospective Iowa Women's Health Study[11,17] investigated dietary factors and UADTC risk in a cohort of 34,691 postmenopausal women, with eventually 169 cases (53 oropharyngeal, 21 laryngeal, 18 nasopharyngeal/salivary, 21 esophageal, and 56 gastric cancer cases), and found that alcohol consumption and smoking increased UADTC risk, whereas intake of fruits and vegetables may reduce risk of UADTC. UADTC subtypes were also investigated, but numbers were too small for statistical testing within individual cancers. Observational studies regarding selenium and HNC risk showed mixed results and mostly had small numbers as well.[18-20] Finally, three prospective cohort studies—with reasonably large case numbers—investigated the association between BMI and HNC risk, but also showed mixed results.[21-23]

In summary, prospective studies regarding alcohol, smoking, dietary factors and HNC often had few HNC cases[10-12], did not inform of specific HNC subtypes[10,12] (or only in very small numbers[11]), combined HNC with esophageal[9,10,12] and gastric[11] cancer into upper aero-digestive tract cancer (UADTC), upper digestive tract cancer (UTDC), or upper aerogastric tract (UAGT) cancers for analyses, or lacked information on smoking duration[13,14], an important facet of smoking behavior. Our large prospective cohort study, with 415 HNC cases (131 OCC, 88 OHPC, and 193 LC) therefore adds significantly to the existing evidence—largely based on case-control studies—regarding the association between lifestyle factors and HNC risk.

Nevertheless, some questions remain unresolved. With regard to BMI, a low BMI (<18.5 kg/m^2) at baseline was inversely associated with HNC risk, whereas for BMI at age 20, a positive rather than inverse association was found. In our study, we did not find indications for reverse causality, but residual confounding might play a role. We concluded that leanness itself is probably not a causal factor in the association with HNC, but we still do not know the exact mechanisms behind the possible association between BMI and HNC risk. This appears to be different than the relationship between BMI and many other cancers, where BMI clearly increases cancer risk.[3] Furthermore, we did not find clear associations for vitamins and carotenoids other than vitamin C on HNC risk. In addition, we found different associations of vitamin supplements and vitamin intake through food on HNC risk. This could be a chance finding or be explained by the small sample size of supplement users, but it might also be that vitamins from supplements simply have different properties than those from whole foods (e.g., vitamin C from vegetables and fruits).

In that view, there are potential—more systemic—mechanisms besides antioxidant properties for the effect of diet and other lifestyle factors on HNC risk which have been less described. First, diet might affect the body's acid-base balance by promoting either acidity or alkalinity, thereby possibly influencing tumor growth.[24,25] A diet rich in vegetables—especially raw vegetables—and fruits may promote alkalinity, which may exert a beneficial effect on HNC risk.[24,25] Furthermore, diet may influence the process of low-grade, chronic inflammation in the body, which is likely to be involved in cancer risk and development as well.[26-31] Chronic inflammation has been widely studied and related to many diseases, including cancer and cardiovascular diseases. Circulating inflammation marker levels have been associated with lung cancer risk in a prospective study.[32] In addition, poor oral hygiene may lead to chronic inflammation (such as periodontitis) as well, and may also be associated with an increased HNC risk.[33] Poor oral hygiene—and possibly other lifestyle factors—might promote the oncogenesis of HNC through chronic inflammation by creating an inflammatory microenvironment that promotes tumor development.[29] Therefore, when we look at the overall picture of dietary factors, smoking, alcohol consumption, oral hygiene, and perhaps also human papillomavirus (HPV), inflammation might even be a common mechanism by which all these factors may influence the risk of developing HNC.

Moreover, poor oral hygiene is also likely related to alcohol consumption and cigarette smoking (and the use of drugs), and might be related to diet. Literature regarding the possible relationship between diet and oral hygiene is scarce, but consumption of certain foods—amongst others vegetables and fruits—might positively influence oral hygiene, possibly as a result of mechanic oral cleaning.[34,35] This might, depending on time of intake, especially be the case in smokers and alcohol drinkers.

To further elaborate on this, it might be promising to closer examine the relationship between dietary patterns (including smoking habits and alcohol consumption) and HNC risk, in addition to the association between food groups and food items on risk of HNC. In everyday practice, people do not just eat separate food items, but generally develop a certain dietary and lifestyle pattern which might have systemic effects (such as described above) on cancer risk. For example, people who eat more vegetables and fruits might have a generally healthier lifestyle, reflecting another dietary pattern than that of people who consume more animal and/or processed foods.[36,37] So far, a dietary pattern with relatively high vegetable and fruit intake and low intake of animal and processed foods has been associated with reduced HNC risk.[36-38] Studying dietary patterns could, therefore, lead to a broader mechanistic view on the relationship between diet and HNC risk.

Finally, in our study, we did not have data on HPV status, nowadays another established risk factor for HNC.[1] None of the prospective studies regarding alcohol, smoking, dietary factors and HNC included HPV data in their research either. Ideally, we would have included such data in our study to adjust for in our analyses in the best possible way, but at the start of our study in 1986, the influence of HPV was not fully known yet. Preferably, all diagnosed oropharyngeal cancer cases during follow-up would have been analyzed on HPV-positivity or –negativity, but gathering this information in our cohort would have been very difficult. For several reasons, however, we argued that the lack of HPV data does probably not significantly influence our findings (see paragraph 'Methodological considerations').

In addition, tumor development is classically divided into three stages: tumor initiation, tumor promotion, and tumor progression. As a carcinogen[1], HPV is likely to be at least involved in tumor initiation. The influence of lifestyle factors might, in that case, subsequently play a role in tumor promotion and progression, by creating a tumor microenvironment that either promotes or inhibits tumor development.[30] For that reason, we would like to speculate that diet may affect HPV-related HNC as well, in a way that HPV may initiate HNC, but nutrition and lifestyle habits may then promote and progress—or inhibit—tumor growth. In that view, our findings may be interesting for HPV-related HNC as well, even though we did not include HPV in our study; this warrants further research.

Differences among head-neck cancer subtypes

In our study, we found consistent differences in associations with risk of HNC subtypes. In general, the risk of OCC and OHPC seemed to be more influenced by alcohol and nutritional factors than LC. This was a consistent pattern (Table 7.1).

How can we explain this differential risk pattern? HNC subtypes are differently located in the head-neck area: OCC and OHPC are part of the aerodigestive tract, whereas LC is part of the respiratory tract. Therefore, specific anatomic subsites come directly in contact with different substances, including potentially HNC-related matters such as carcinogens (e.g., ethanol) and antioxidants (e.g., vitamin C). These substances may have an indirect, systemic effect (i.e., they become available in the body after being metabolized and subsequently act in numerous processes across the body, e.g., acetaldehyde, the main metabolite of ethanol), but they may also exert their effect on HNC carcinogenesis through direct exposure to tissue in the head-neck area. The differential risk among HNC subtypes may therefore be explained by the larynx having

the least direct exposure to alcohol and food substances compared with the oral cavity and pharynx.[4,6] In addition, the oral cavity seems to be the least susceptible to the effects of cigarette smoking.[39,40] This leads us to another possible explanation for the HNC risk pattern regarding cigarette smoking, which could be the aerodynamics of respiratory flow in the upper airway. This flow changes from predominantly laminar in the oral cavity to predominantly turbulent in the larynx, which may result in the larynx and pharynx having a higher exposure to inhaled air—and thus to cigarette smoke—than the oral cavity. Differences in associations among HNC subtypes could thus be explained by different exposures due to the location of the tumor.

Regarding selenium, strongest inverse associations were found for OHPC and LC, which in our study were the subtypes also strongest associated with cigarette smoking. Our findings might point to the possibility that, despite thorough adjustment for confounding by smoking, there might still be residual confounding. The fact that most associations were substantially stronger in age- and sex-adjusted analyses than in multivariable-adjusted analyses further implies this possibility. However, it may also indicate that the effect of selenium on HNC risk is associated with smoking. We did show that toenail selenium was inversely associated with HNC risk especially in current smokers, but without a statistically significant interaction, perhaps due to a lack of power. It might be biologically plausible that the effect of selenium on HNC risk is associated with smoking, since selenium is an antioxidant and counteracts the oxidative stress caused by smoking.[3] This would, however, raise the question why we did not find a similar pattern among HNC subtypes with regard to vegetable and fruit consumption, and vitamin intake.

Methodological considerations

Our study has several important strengths, as well as some limitations, and there are interesting methodological points to consider.

Important strengths of our study are the prospective nature and duration and completeness of follow-up. This makes recall bias, reversed causation and loss to follow-up bias unlikely. Given our large number of cases, we were able to examine the effect of lifestyle factors not only on risk of HNC overall, but also on the risk of HNC subtypes. Furthermore, we had the ability to adjust for confounders extensively, which is vitally important since cigarette smoking and alcohol consumption are main risk factors for HNC.

A possible limitation of our study is the single measurement of exposure data. Although validation and reproducibility studies showed good results[41,42], the FFQ may have provided only moderately accurate estimates of food intake and other lifestyle habits. We believe the FFQ was given to participants in an age group with stable dietary habits at baseline, but it is nevertheless possible that participants changed their dietary habits since 1986. Although cigarette smoking and alcohol consumption were extensively addressed, it is also possible that participants changed their smoking habits and/or habitual alcohol intake at some point during follow-up. All this may have led to bias due to random (or non-differential) misclassification of exposure, possibly resulting in an underestimation of the effects of the studied factors on HNC risk. It may also have led to bias when adjusting for smoking as a confounder, but we did take this into account in our analyses by investigating and correcting for an interaction of smoking status with time if necessary (see Chapter 3).

We cannot exclude the presence of some residual confounding by smoking and/or alcohol, although we had detailed information about alcohol consumption and cigarette smoking and were therefore able to thoroughly adjust for these confounders. Residual confounding may also have occurred due to other potential confounders, such as physical activity and family history of HNC, but as we investigated many of these, we presume this to be limited.

Furthermore, although our study includes a large number of cases, a lack of power is a possible explanation for finding non-significant results for some associations and the tests for heterogeneity. In stratified analyses, we mostly did not find a statistically significant interaction with regard to cigarette smoking status or alcohol consumption. However, analyses included only few cases among never smokers and never drinkers, and there may have been a lack of power to detect a significant interaction. Finally, our categorization of alcohol consumption may have affected results of the (interaction) analyses, but we tested several options and used the best possible categorization for each analysis.

Our study lacks information on HPV infection, which has been associated with HNC risk.[1,43-45] HPV infection is associated with HNC risk[43,46], but mainly with OHPC, in particular tonsil cancer and cancer of the base of the tongue. According to rates in our university medical center (personal communication of prof. dr. B. Kremer), only 25% of the diagnosed and treated oropharyngeal cancers between 1997 and 2003 were HPV-positive (all oropharyngeal cancer cases have been analyzed by p16-immunostaining and HPV16-specific fluorescence in situ hybridization (FISH), and—if FISH was negative—HPV-specific polymerase chain reaction). Moreover, the role of HPV in HNC carcinogenesis is mainly of importance in young HNC patients, and has increased since

1990[45,47,48], whereas our participants were aged 55-69 years at baseline in 1986. For these reasons, we assume that the number of HPV-associated HNC cases in our cohort is low, and we expect potential bias due to possible misclassification to be very limited.

We were not able to include the use of drugs and oral hygiene in our study. As mentioned before, we speculate that oral hygiene may play a role in the development of HNC, especially OCC, and it might in that view be interesting to include this factor in future studies.[33,49]

Future studies might be able to address and elaborate on some of the possible limitations we had in our study, as will be described in the last paragraph.

Implications for public health

With regard to HNC, it has been estimated that up to half of these cancers are preventable by appropriate diets and associated lifestyle factors.[3] As we wrote in Chapter 1, treatment decisions are complex, often with side effects resulting in significant morbidity with regard to basic functions such as speaking, swallowing, and breathing. Our results strengthen the evidence that lifestyle factors importantly influence HNC risk, and health guidelines may help support and encourage people to act accordingly.

Furthermore, the lifestyle factors that influence the risk of HNC and HNC subtypes also influence risk of other diseases, such as cardiovascular diseases (CVD) and other types of cancer. Cigarette smoking and alcohol consumption are known risk factors for CVD and many types of cancer. Dietary factors, like vegetable and fruit intake, importantly influence risk of other diseases as well. The same applies for overweight and obesity.

In addition to HNC, the ongoing WCRF systematic review of worldwide research[50] shows that about a third of the most common cancers are preventable through a nutritious diet, maintaining a healthy weight and regular physical activity. The WCRF therefore set up Cancer Prevention Recommendations, which are the result of years of research in this field. Where applicable, our results support these recommendations (Table 7.2).

Ultimately, the goal of health guidelines is to decrease the burden of disease and mortality due to preventable causes. In that perspective, our results underline that prevention—by making healthy lifestyle choices—is a promising strategy in HNC.

Table 7.2. WCRF Cancer Prevention Recommendations[50]

1	Be as lean as possible within the normal range of body weight.
2	Be physically active as part of everyday life, for at least 30 minutes every day.
3	Limit consumption of energy-dense foods (foods high in fats and/or added sugars and/or low in fibre) and avoid sugary drinks.
4	Eat mostly foods of plant origin; eat more of a variety of vegetables, fruits, wholegrains, & pulses such as beans.
5	Limit intake of red meat & avoid processed meat.
6	Limit alcoholic drinks. If consumed at all, limit alcoholic drinks to 2 for men and 1 for women a day. This recommendation takes into account that there is a likely protective effect for coronary heart disease.
7	Limit consumption of salt & avoid mouldy grains and cereals
8	Don't use supplements to protect against cancer; aim to meet nutritional needs through diet alone.
9	It is best for mothers to breastfeed exclusively for up to 6 months and then add other liquids & foods.
10	After treatment, cancer survivors should follow the recommendations for cancer prevention.

Concluding remarks

The general aim of this thesis was to study and further establish the existing evidence regarding the association between several lifestyle factors and the risk of developing HNC and HNC subtypes.

Our results confirm the principal role of alcohol consumption and cigarette smoking in HNC carcinogenesis. As we hypothesized, the risk of HNC was higher in participants with a low intake of vegetables and fruits and vitamin C, and those with low levels of toenail selenium. No clear associations were, however, found for intake of vitamin E and carotenoids. In addition, the risk of HNC was lower in participants with a high BMI at study baseline, but not at age 20; we concluded that leanness itself is probably not a causal factor in the association with HNC. Associations of the studied lifestyle factors with HNC risk were not clearly modified by smoking and alcohol consumption in our study, although we expected otherwise. Finally, we did find a consistent pattern in differences in risk associations among the HNC subtypes OCC, OHPC, and LC: LC risk seemed to be more smoking related, whereas alcohol and nutrition appeared to have a stronger influence on risk of OCC.

In conclusion, lifestyle factors, including nutrition, significantly influence HNC risk. As prospective data are scarce, this cohort study considerably contributes to establish in which extent lifestyle factors are associated with risk of HNC overall and especially HNC subtypes. Nevertheless, there are specific items that seem to be important to address in future studies regarding the etiology of HNC.

Recommendations for future research

Future research is warranted for further clarifications of the possible mechanisms involved regarding lifestyle factors and HNC risk, and to confirm our results, especially associations between vitamin and carotenoid intake, toenail selenium status, BMI and HNC risk. We found a differential risk between toenail selenium and HNC risk in men and women, but it is unclear what reasons there might be behind this possible difference. It would therefore be interesting to further study this association and the possible mechanisms behind it.

Since our results indicate clear association differences among the most common HNC subtypes, in future research these tumor groups would ideally be split up, as there likely are different mechanistic pathways involved. We especially recommend studying OCC and LC separately, if possible. In our study, OHPC risk mostly resembled OCC risk, but it requires more research to put this tumor group in perspective, perhaps more correctly, and perhaps even further split up into oropharyngeal and hypopharyngeal cancer. It would, however, require large case-numbers of OCC, OHPC, and LC to further study and clarify these associations pattern we found.

We also recommend including HPV-status in future studies regarding HNC etiology. As HPV-related HNC incidence has been on the rise since 1990, we believe that future prospective studies need to incorporate HPV in (the set-up of) their study. Although still a minority of HNC is HPV-related, it is important to include HPV in future studies in order to obtain the most comprehensive view on HNC risk.

We did not find clear interaction patterns between alcohol consumption, cigarette smoking and other lifestyle factors, but that might have to do with a lack of power, even though our study is one of the largest prospective studies so far. To examine these interactions therefore warrants further study, in particular with larger case-numbers and thus more study power, or possibly in a pooled analysis of prospective studies. It might, in that view, also be interesting to examine the RRs of HNC for smokers among non-drinkers and for drinkers among non-smokers with large case-numbers. However, as case numbers for these subgroups are generally very small, this item will probably be difficult to address.

Finally, there are other interesting factors—though outside the scope of this thesis—to include in future research to broaden the proverbial 'HNC horizon'. First, it might be interesting to not only further investigate dietary factors per se, but also in terms of dietary patterns, as we described earlier. Furthermore, psychosocial factors[51] have been studied in relationship to cancer risk, but this association remains unclear and controversial. According to the US National Cancer Institute[51], the body responds

to physical, mental, or emotional stress by releasing stress hormones that increase blood pressure and raise blood sugar levels. Research has shown that people who experience chronic stress can have digestive problems, fertility problems, a weakened immune system, and are also more prone to viral infections. Given these physical responses, chronic stress may also lead to the previously described process of chronic inflammation. It might therefore be interesting to explore whether psychosocial health may contribute to HNC risk, and—just to speculate in this thesis—in the future perhaps be able to model HNC risk in an integral biopsychosocial model. Also, given the importance of lifestyle factors in HNC risk, gene-environment interactions might also be of interest to explore with respect to HNC risk, with genes relevant in HNC etiology. Lastly, it might also be interesting to study the influence of lifestyle factors after HNC diagnosis with regard to HNC survivorship, and find out if it might literally never be too late for lifestyle changes.

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Summary

Head-neck cancer (HNC) is the seventh most common type of cancer in the world and Europe. Cancers located in the oral cavity, pharynx, and larynx are the most frequent HNC subtypes, and they largely share the same risk factors (**Chapter 1**). Treatment decisions are complex, with considerable early and late side effects. In addition, survival rates are variable and average around 50-60 per cent at 5 years, depending on the type or anatomic sublocalization of HNC.

It has been estimated that up to half of HNC cases are preventable by lifestyle factors and appropriate diets. Especially since treatment options are limited and survival rates poor, the focus on prevention of HNC—by lifestyle—is of great importance. Evidence with regard to lifestyle and dietary factors and HNC risk, in particular risk of HNC subtypes, is limited and mainly based on case-control studies; prospective data are scarce. Therefore, the general aim of this thesis was to study the association between several lifestyle factors and the risk of developing HNC and HNC subtypes—i.e., oral cavity cancer (OCC), oro-/hypopharyngeal cancer (OHPC), and laryngeal cancer (LC)—in a large prospective cohort study.

We investigated the associations between alcohol consumption, cigarette smoking, consumption of vegetables and fruits, vitamin and carotenoid intake, toenail selenium status, and body mass index (BMI) and risk of HNC and HNC subtypes within the Netherlands Cohort Study on diet and cancer (NLCS). The NLCS was initiated in September 1986 and includes 120,852 participants, 58,279 men and 62,573 women, aged 55-69 years from 204 Dutch municipal population registries. At baseline, all participants completed a self-administered questionnaire on dietary habits and other cancer risk factors, such as smoking, alcohol consumption, and anthropometry. The 11-page questionnaire included a 150-item food frequency questionnaire that focused on habitual food consumption during the year preceding the start of the study. In addition, about 75% of the participants collected toenail clippings.

For the NLCS, the case-cohort design was used for efficiency in data processing, follow-up and analyses. Cases were derived from the entire cohort, whereas the number of person-years at risk for the entire cohort was estimated using a subcohort of 5,000 people, randomly sampled from the total cohort at baseline and followed up for vital status information. The entire cohort is being monitored for cancer incidence by annual record linkage to the Netherlands Cancer Registry, and the nationwide network and registry of histo- and cytopathology in the Netherlands. After 20.3 years of follow-up, 415 incident HNC (131 OCC, 88 OHPC, 3 oral cavity/pharynx unspecified or overlapping, and 193 LC) cases were available for analysis.

In **Chapter 2**, we studied the association between alcohol consumption, cigarette smoking and HNC risk. Alcohol consumption and cigarette smoking were strongly,

independently associated with an increased HNC risk. In addition, we found a positive, multiplicative interaction between both factors regarding HNC risk. The strength of these associations, however, differed among subtypes; OCC was most strongly associated with alcohol consumption but most weakly with cigarette smoking, whereas LC was not statistically significantly associated with alcohol consumption.

Next, we found that total vegetable and fruit consumption were related to a decreased risk of HNC overall and all HNC subtypes (**Chapter 3**), with the strongest inverse associations for OCC. Total vegetable consumption was inversely associated with risk of HNC overall as well; associations with HNC subtypes were also inverse and were largely similar for OCC, OHPC, and LC. Total fruit consumption was associated with reduced risks of HNC overall and all subtypes; the strongest associations were with OCC. Associations between vegetable and fruit consumption and HNC risk were not clearly modified by cigarette smoking status or alcohol consumption.

In **Chapter 4**, we examined associations of vitamin and carotenoid intake with HNC risk. Vitamin C intake through diet was significantly associated with a decreased risk of HNC overall, OCC and OHPC. No clear associations were found for other nutrients and vitamin supplementation; however, most point estimates showed possible protective effects. Furthermore, the association between vitamin E intake and the risk of HNC overall was modified by alcohol consumption, with lower risks in alcohol abstainers.

In **Chapter 5**, we investigated selenium, a trace element present in food which is likely to be involved in cancer risk. A low toenail selenium level was related to an increased HNC risk in our study. Among HNC subtypes, this inverse association was strongest for OHPC and LC. The association between toenail selenium and risk of HNC overall was considerably stronger among men than women. Although there was no clear effect modification by cigarette smoking or alcohol consumption in our study, the inverse association we found was most explicit among current smokers.

Lastly, we found an inverse association between BMI at baseline and HNC risk (**Chapter 6**): being underweight (BMI <18.5 kg/m^2) at baseline was related to an increased HNC risk. Among HNC subtypes, this association was strongest for OCC and OHPC. For BMI at age 20, on the other hand, we found a positive rather than inverse association. The association between change in BMI since the age of 20 years and HNC risk appeared to be inverse again. There was effect modification by alcohol consumption in our study, with the lowest risks of HNC overall for BMI at baseline, BMI at age 20, and change in BMI in non-drinkers.

We generally discuss our findings and put them into perspective in **Chapter 7**. Our results strengthen the evidence that lifestyle factors importantly influence HNC risk. Alcohol consumption and cigarette smoking play a principal role in HNC carcinogenesis.

Furthermore, a low intake of vegetables, fruits and vitamin C were associated with an increased HNC risk, as well as low levels of toenail selenium. Intake of a vitamin C supplement, vitamin E and carotenoids, on the other hand, do not seem to influence HNC risk. Regarding BMI, we concluded that leanness itself is probably not a causal factor in the association with HNC; HNC risk was lower in participants with a high BMI at study baseline, but not at age 20. Associations of the studied lifestyle factors with HNC risk were not clearly modified by smoking and alcohol consumption.

Regarding HNC subtypes, we found consistent differences in risk associations. In general, the risk of OCC and OHPC seemed to be more influenced by alcohol and nutritional factors than LC. Therefore, in future research regarding the etiology of HNC, these tumor groups would ideally be split up. Future research is also warranted for further clarifications of the possible mechanisms involved regarding lifestyle factors and HNC risk, and to confirm our results. Furthermore, we recommend including HPV status in future studies. In conclusion, the results described in this thesis underline that prevention—by making healthy lifestyle choices—is a promising strategy in HNC.

Samenvatting

Hoofd-halskanker (HHK) is wereldwijd en in Europa de zevende meest voorkomende vorm van kanker. Kanker van de mondholte, keelholte en het strottenhoofd zijn de meest voorkomende subtypen van HHK en kennen grotendeels dezelfde risicofactoren (**Hoofdstuk 1**). De vraag hoe HHK patiënten het best behandeld kunnen worden is vaak complex, omdat behandeling nadelige gevolgen kan hebben voor diverse lichaamsfuncties, op zowel korte als lange termijn. De gemiddeld 5-jaarsoverleving voor HHK is 50-60%; afhankelijk van het type of anatomische sublokalisatie van HHK verschillen de overlevingskansen aanzienlijk.

Er wordt geschat dat de helft van alle gevallen van HHK voorkómen kan worden door leefstijlfactoren, waaronder een gezond voedingspatroon. Zeker omdat behandelingsopties beperkt en overlevingskansen gering zijn, is aandacht voor de preventie van HHK door leefstijl daarom van groot belang. Het bewijs voor het verband tussen leefstijl, voedingsfactoren en het risico op HHK, met name het risico op HHK subtypen, is echter beperkt en voornamelijk gebaseerd op case-control studies; prospectieve gegevens zijn schaars. Het doel van dit proefschrift is dan ook het verband tussen verschillende leefstijlfactoren en het risico op HHK en HHK subtypen—i.e., kanker van de mondholte (MK), keelholte (de oro- en hypofarynx: OHK) en het strottenhoofd (de larynx: LK)—te bestuderen in een grote prospectieve cohortstudie.

We hebben het verband tussen alcoholgebruik, roken, de consumptie van groente en fruit, de inname van vitaminen en carotenoïden, seleniumwaarden in teennagels, body mass index (BMI) en het risico op HHK en HHK subtypen onderzocht binnen de Nederlandse Cohort Studie naar voeding en kanker (NLCS). De NLCS werd opgericht in september 1986 en bestaat uit 120.852 deelnemers, 58.279 mannen en 62.573 vrouwen, in de leeftijd van 55-69 jaar uit 204 gemeentelijke bevolkingsregisters. Bij aanvang van de studie hebben alle deelnemers een vragenlijst over voedingsgewoonten en andere kankerrisicofactoren, zoals roken, alcoholgebruik en lichaamsgewicht, ingevuld. Deze 11 pagina's tellende vragenlijst bestond onder meer uit een voedingsvragenlijst van 150 items, gericht op de gebruikelijke consumptie van voedingsmiddelen in het jaar voorafgaand aan de start van de studie. Daarnaast heeft ongeveer 75% van de deelnemers een teennagelmonster aangeleverd.

In de NLCS gebruiken we voor de analyses het zogeheten case-cohort design vanwege efficiëntie in dataverwerking en follow-up. Cases zijn afkomstig uit het gehele cohort, terwijl het aantal persoonsjaren 'at risk' voor het hele cohort wordt geschat aan de hand van een subcohort. Dit subcohort, waarvan ook de vitale status wordt opgevolgd, is een willekeurige subgroep van 5.000 deelnemers uit het totale cohort bij baseline. De incidentie van kanker wordt in het gehele cohort opgevolgd door middel van jaarlijkse koppeling met de Nederlandse Kankerregistratie en het landelijk netwerk

en register van histo- en cytopathologie (PALGA). Na ruim 20 jaar follow-up waren 415 incidente gevallen van HHK (waarvan 131 MK, 88 OHK, 3 mond-/keelholte niet nader gespecificeerd of overlappend en 193 LK) beschikbaar voor analyse.

In **Hoofdstuk 2** hebben we het verband tussen alcoholgebruik, roken en het risico op HHK bestudeerd. Zowel alcoholgebruik als roken waren sterk en onafhankelijk van elkaar geassocieerd met een verhoogd risico op HHK. Daarnaast vonden we een positieve, multiplicatieve interactie tussen beide factoren met betrekking tot HHK risico. De sterkte van deze associaties verschilde echter tussen subtypen; MK was het sterkst geassocieerd met de consumptie van alcohol, maar het zwakst met het roken van sigaretten, terwijl LK niet statistisch significant geassocieerd was met alcoholgebruik.

De totale consumptie van groente en fruit was gerelateerd aan een verlaagd risico op HHK in het algemeen en alle HHK subtypen (**Hoofdstuk 3**), met de sterkste inverse verbanden voor MK. Totale groenteconsumptie was eveneens invers geassocieerd met HHK risico; ook verbanden met HHK subtypen waren invers en grotendeels vergelijkbaar voor MK, OHK en LK. Totale fruitconsumptie was geassocieerd met een verminderd risico op HHK in het algemeen en alle subtypen, met de sterkste verbanden voor MK. Associaties tussen groente- en fruitconsumptie en HHK risico veranderden niet duidelijk in verschillende categorieën van roken of alcoholgebruik.

In **Hoofdstuk 4** onderzochten we het verband tussen de inname van vitaminen en carotenoïden en het risico op HHK. Inname van vitamine C via de voeding was significant geassocieerd met een verlaagd risico op HHK in het algemeen, MK en OHK. We vonden geen duidelijke associaties met HHK risico voor andere vitaminen en carotenoïden dan wel het gebruik van vitaminesupplementen, hoewel de meeste puntschattingen mogelijk beschermende effecten lieten zien. De relatie tussen vitamine E inname en het risico op HHK werd gemodificeerd door alcoholgebruik, met lagere risico's voor alcoholonthouders.

In **Hoofdstuk 5** onderzochten we selenium, een sporenelement in de voeding dat waarschijnlijk betrokken is bij het risico op kanker. In onze studie was een laag seleniumgehalte in teennagels geassocieerd met een verhoogd risico op HHK. Onder HHK subtypen was dit inverse verband het sterkst voor OHK en LK. Het verband tussen teennagelselenium en HHK risico was aanzienlijk sterker onder mannen dan onder vrouwen. Hoewel onze studie geen duidelijke effect modificatie door het roken van sigaretten of het gebruik van alcohol liet zien, was de inverse associatie die we gevonden hebben wel het meest expliciet onder huidige rokers.

Ten slotte hebben we een invers verband tussen BMI bij aanvang van de studie (‘BMI bij baseline’) en het risico op HHK gevonden (**Hoofdstuk 6**): het hebben van

ondergewicht (BMI <18.5 kg/m^2) bij baseline was gerelateerd aan een verhoogd HHK risico. Onder HHK subtypen was dit inverse verband het sterkst voor MK en OHK. Voor BMI op 20-jarige leeftijd vonden we daarentegen juist een positief verband. De associatie tussen verandering in BMI sinds de leeftijd van 20 jaar en HHK risico leek weer invers te zijn. Er was effect modificatie door alcoholgebruik in onze studie, met de laagste risico's op HHK in het algemeen voor BMI bij baseline, BMI op 20-jarige leeftijd en verandering in BMI onder niet-drinkers.

In **Hoofdstuk 7** hebben we onze bevindingen in het algemeen besproken; daarnaast hebben we geprobeerd deze in perspectief te plaatsen. Onze resultaten versterken het bewijs dat leefstijlfactoren in belangrijke mate van invloed zijn op het risico HHK te ontwikkelen. Alcoholgebruik en het roken van sigaretten spelen daarin een hoofdrol. Verder zijn een lage inname van groente, fruit en vitamine C, evenals een lage teennagelseleniumwaarde, geassocieerd met een verhoogd risico op HHK. Het gebruik van een vitamine C supplement, evenals de inname van vitamine E en carotenoïden, lijkt het risico op HHK daarentegen niet te beïnvloeden. Ten aanzien van BMI hebben we geconcludeerd dat het hebben van een laag lichaamsgewicht (BMI <18.5 kg/m^2) op zichzelf waarschijnlijk niet een oorzakelijke factor is in de associatie met HHK. Het risico op HHK was weliswaar lager onder deelnemers met een hoge BMI bij aanvang van de studie, maar was níet lager onder deelnemers met een hoge BMI op 20-jarige leeftijd. Associaties tussen de onderzochte leefstijlfactoren en het risico op HHK werden niet duidelijk gemodificeerd door roken en alcoholgebruik.

Met betrekking tot HHK subtypen hebben we consistente verschillen in risico associaties gevonden. In het algemeen lijkt het risico op MK en OHK meer beïnvloed te worden door alcohol en voeding dan het risico op LK. Mede op basis van onze studie zouden we daarom willen aanbevelen om bij vervolgonderzoek gericht op de etiologie van HHK deze tumorgroepen te splitsen. Daarnaast is toekomstig onderzoek belangrijk voor verdere verheldering van de mogelijke mechanismen die betrokken zijn bij het verband tussen leefstijlfactoren en het risico op HHK, alsook om onze studieresultaten te bevestigen. Verder raden we het meenemen van HPV status in toekomstige studies aan. We kunnen concluderen dat de in dit proefschrift beschreven resultaten onderstrepen dat het maken van gezonde leefstijlkeuzes een veelbelovende strategie ter preventie van HHK is.

Valorization

By law, Dutch universities have three main tasks: to educate at an academic level, to conduct scholarly research, and to ensure that research findings impact society. Valorization is the term that governmental and university policymakers use to denote this process of 'translating academic wisdom to societal benefit'. This chapter outlines the valorization potential of the research findings presented in this thesis.

From scientific to societal value

Knowledge valorization involves the process of creating societal and/or economic value from scientific knowledge. How could this dissertation's key finding—that lifestyle factors importantly influence HNC risk—have a societal impact that goes beyond well-cited publications?

It has been estimated that up to half of HNC cases are preventable by appropriate diets and associated lifestyle factors.[1] The results described in this thesis underline that prevention—by making healthy lifestyle choices—is a promising strategy in HNC. Alcohol consumption and cigarette smoking are main risk factors for HNC and strongly increase HNC risk. Furthermore, we clearly showed that other lifestyle factors significantly influence HNC risk as well. Vegetable and fruit consumption, as well as vitamin C intake, were significantly associated with a decreased HNC risk. Finally, a low toenail selenium status was associated to an increased HNC risk, as well as being underweight (BMI <18.5 kg/m^2) at baseline. In conclusion, lifestyle factors, including nutrition, significantly influence HNC risk. Especially since treatment options are limited and survival rates poor, the focus on prevention of HNC—by lifestyle—is of great importance.

Furthermore, the lifestyle factors that influence HNC risk also influence risk of other diseases, such as cardiovascular diseases (CVD) and other cancer types. The World Cancer Research Fund systematic review of worldwide research[2] shows that not only many HNC cases, but also about a third of the most common cancers are preventable through diet, a healthy weight, and physical activity. Cigarette smoking and alcohol consumption are known risk factors for CVD and many types of cancer. Dietary factors, like vegetable and fruit intake, importantly influence risk of other diseases as well. The same applies for overweight and obesity.

In view of the above, our findings may have an impact in several ways. We will describe what merit our results might have for public health, both nationally and internationally; for health care, in general and for the individual; and finally for education and future research regarding lifestyle factors and HNC risk.

Translating research into (daily) practice: dietary recommendations?

Together with prior and future research findings, our findings could jointly result in useful outcomes such as dietary recommendations. Our results strengthen the evidence that lifestyle factors importantly influence HNC risk, and health guidelines may help support and encourage people to act accordingly.

The Cancer Prevention Recommendations set up by the World Cancer Research Fund (WCRF)[2] are an example of such guidelines (Chapter 7, Table 7.2, and Figure 1 below). These recommendations are the result of years of ongoing research, known as the WCRF Continuous Update Project (CUP)[3]. As part of the CUP, global scientific research—randomized controlled trials and cohort studies—on how diet, physical activity, and weight affect cancer risk and survival is being systematically reviewed. Among experts, the WCRF CUP it is a trusted, authoritative scientific resource, which underpins current guidelines and policy for cancer prevention.[3]

Our findings directly contribute to the CUP with regard to HNC, thereby proving their value from scientific results to practical recommendations. A review of the Cancer Prevention Recommendations is expected in 2017; where applicable, our results support the current recommendations. The American Institute for Cancer Research (AICR)[4] is part of the WCRF and thus disseminates the same recommendations for cancer prevention. These recommendations enable everyone, from policy makers to members of the public, to have access to the most up-to-date information on how to minimize the risk of developing cancer.

Eventually, our findings may also contribute to the WCRF NOURISHING framework[5]. This framework helps policymakers worldwide to identify where action is needed to promote healthy diets and to reduce obesity and other non-communicable diseases, including cancer. As a result, many different policy actions have already been taken, a perfect example of valorization. Key domains are food environment (NOURIS), food system (H), and behavioral change (ING). Examples of areas where governments need to take action are to offer healthy foods and set standards in public institutions and other specific settings; to set incentives and rules to create a healthy retail and food service environment; to harness food supply chain & actions across sectors to ensure coherence with health; to inform people about food & nutrition through public awareness; to give nutrition advice and counselling in health care settings; to give nutrition education and skills.[5]

Figure 1. Infographic launched by WCRF[2] for World Cancer Day: 'Empowering people to make healthy choices'

In addition to the WCRF and AICR, other public health organizations set up dietary recommendations to lower disease—including cancer—risk as well, based on sound scientific results which in the future might include ours. The World Health Organization (WHO) acts globally to help people eating healthier and governments enabling people to do so.[6] The Health Council of the Netherlands (Gezondheidsraad)[7] advises the Dutch authorities on health recommendations. Based on dietary guidelines of the Health Council[8], the Netherlands Nutrition Centre[9] (NNC; Voedingscentrum) recently released their latest version of the so-called Food Pyramid, in Dutch better known as 'De Schijf van Vijf'[10] (Figure 2). The NNC states that healthy food is an essential part of a healthy lifestyle, and that healthy eating habits are often the best remedy for many of today's most common health problems as well as to prevent chronic diseases. The NNC seeks to explain the relevance of scientific knowledge and translate it into understandable and practical dietary guidelines. Lastly, the NNC puts the issue of healthier and more sustainable nutrition on the agendas of the food industry and consumers, as well as in politics and the media.[9]

The Food Pyramid is a practical information tool used by the NNC in order to encourage consumers to develop and maintain healthy eating habits, and to prevent obesity. Scientific results are thus being translated into a tool for daily practice.[9,10] Key messages of the Food Pyramid are to eat lots of vegetables, fruits, and wholemeal products; less meat, and more legumes, nuts, eggs, or tofu instead; and to limit consumption of processed foods, which generally contain a lot of sugar, salt and unhealthy fats.[9,10] Where applicable, the current recommendations of the Food Pyramid are in concordance with our study results.

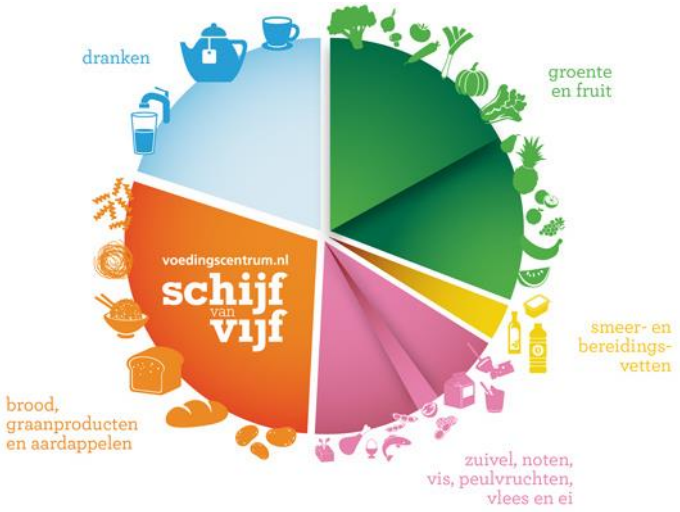

Figure 2. The Food Pyramid, released March 2016[10]

Other possible implications for public health and healthcare

In addition to a possible contribution to health recommendations and dietary guidelines, our results have been considered relevant and drawn attention in various ways. We communicated our study findings to various audiences, with possible implications for public health, healthcare, and future research.

In 2012, 2013 and 2015, our results were shown at the Dutch General Meeting for Otorhinolaryngology, with an audience consisting mostly of clinicians. In 2013, our study results were presented to a public of largely clinicians as well at the 4th World Congress of the International Academy of Oral Oncology in Greece. Furthermore, our study was one of the topics at the Rank Prize Funds mini-symposium on Alcohol and Nutrition in the United Kingdom in 2011. These Funds have as their objectives the advancement and promotion for the public benefit of knowledge, education and

learning. Finally, our study findings have been presented at the Dutch Epidemiology Meeting in 2012. An invitation to speak for dentists, general practitioners and other clinicians at a conference entitled 'Nutrition–healthy living!' in November 2016 has been accepted.

Furthermore, our findings were highlighted in the Dutch media. In 2014, our results concerning alcohol consumption, cigarette smoking and HNC risk were published in Metro, a Dutch newspaper.[11] News about the inverse association between vegetables and fruit intake and HNC risk appeared online and in the newspaper Het Parool.[12-14] Finally, our findings regarding vitamin C and HNC risk were broadcasted by TV Maastricht, a local television channel, in August 2015, and appeared on the known Dutch news website NU.nl.[15,16] Hopefully, the announcement of our results at several conferences and in the media has contributed to creating awareness regarding lifestyle factors and HNC among both clinicians and the public.

Treatment decisions regarding HNC are complex, often with side effects resulting in significant morbidity with respect to basic functions such as speaking, swallowing, and breathing, as we wrote in Chapter 1. For these reasons, we believe our results might contribute to individual health care. We hope our findings will eventually lead to a decrease in morbidity and mortality due to HNC that could possibly have been prevented. Through (future) health guidelines and increased awareness, we ultimately aim for the preservation of health and well-being of individual persons. The findings described in this thesis may also impact the community as a whole, by affecting the economic burden of health care provided to HNC patients as well as productivity loss at work due to health problems. Lastly, since the beneficial effects of a healthy lifestyle go beyond those respecting HNC risk and also influence other common (chronic) diseases such as cancer, CVD, and diabetes, the implications of a healthy lifestyle for health, well-being and healthcare may be even more far-reaching.[2]

As mentioned before, increased awareness about the relationship between lifestyle factors and health among both the public and clinicians is important. Ideally, information—for example by dietary guidelines—on how to eat and live healthy should be something to talk about in healthcare, at the general practitioner as well as in hospitals. Lately, there has been an increased interest in the relationship between lifestyle, nutrition, health, and disease prevention among public and clinicians, and there have been promising initiatives. Our own academic hospital, the Maastricht University Medical Center+, is a good example of this: its focus has been aimed at 'Healthy Living', including prevention, for a while now.[17] Furthermore, together with the foundation Voeding Leeft[18], two Dutch general practitioners established the

Vereniging Arts en Voeding[19] to increase national awareness about diet and health among clinicians, and encourage them to use this knowledge in their work. In addition, they plead for more health education and promotion, starting with more attention for nutrition, in medical studies. Another recent initiative is the release of the website Sick of Smoking[20], which is supported by patients, clinicians, midwifes and others, and aims to increase public debate regarding smoking. Earlier, two Dutch pulmonologists started the Stichting Rookpreventie Jeugd[21], helped set up the website TabakNee[22], and held a TEDx talk[23] in their mission to stop people from smoking in 2013.

In view of the foregoing, however, it has to be noted that it is not easy to put health recommendations into practice for everyone. Especially since we live in an environment that constantly offers challenges in making healthy lifestyle choices, with unhealthy foods and products easily available, changing dietary or smoking habits remains difficult. Ideally, healthy foods and products should be plenty available in public institutions such as schools or railway stations. Thus, as described earlier regarding the WCRF NOURISHING framework[5], food environment, food system, and behavioral change are important subjects for policy action.

Precaution and future research

For some lifestyle factors, the evidence is firmer than others. As one of the largest cohort studies on lifestyle factors and HNC risk so far, our findings regarding alcohol consumption, cigarette smoking, and intake of vegetables and fruits and HNC risk confirm and strengthen the existing evidence. In addition, these findings fit current—and probably add to future—dietary recommendations.

For other factors, however, we believe we should be more conservative before jumping to conclusions with regard to lifestyle recommendations or any clinical advice. We believe more research is warranted regarding the association between selenium and HNC risk, as described in Chapter 5. We also recommend future research to investigate the underlying mechanisms and to confirm our results regarding the association between intake of vitamins and carotenoids and HNC risk. Finally, future studies are warranted for further clarifications of the possible mechanisms regarding BMI and HNC risk. Other recommendations for further research have been described in Chapter 7.

Conclusion

This chapter shows how the scientific findings described throughout this thesis might contribute to public health, healthcare, and future research and education. Our results could be used by policymakers, clinicians, researchers, and—last but not least—the public, to increase health and well-being and—ultimately—attain a decrease in the burden of disease and mortality due to preventable causes. In that view, our results may eventually lead to lower societal costs by reducing the health care burden related to HNC, as well as costs associated with productivity losses. Furthermore, our results provide important new leads for further research regarding lifestyle factors and HNC that could affect (future) health care and health guidelines as well. In conclusion, our results underline that prevention is a promising strategy in HNC and can be translated into societal value in many ways.

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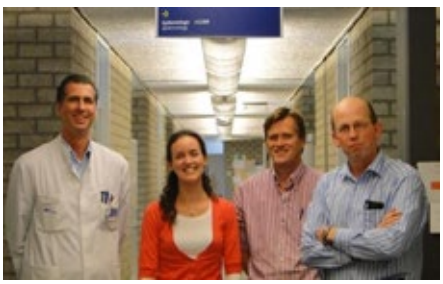

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Denise

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Curriculum Vitae

Denise Maasland was born on August 11, 1983, in Heerlen, the Netherlands. After completing secondary school (Gymnasium; Strabrecht College, Geldrop) in 2001, she studied Health Sciences (2001-2006) and Medicine (2002-2009) at Maastricht University.

Regarding Health Sciences, Denise wrote her bachelor's thesis 'The role of growth factors in the development of breast cancer: possibilities for prevention' in 2004. She performed her master's internship 'The Role of Angiogenesis in B-cell lymphoma' at the Department of Pathology (Maastricht University Medical Center+). In May 2006, Denise obtained her master's degree in Health Sciences, with a specialization in Biological Health Sciences.

During medical school, Denise did her elective in pediatric oncology (The Royal Children's Hospital/The Children's Cancer Center, Melbourne, Australia) and performed her senior internship in radiation oncology (Catharina Hospital, Eindhoven). After obtaining her medical degree in August 2009, Denise worked as a doctor in youth public health (Jeugdgezondheidszorg; Public Health Service (GGD), Eindhoven) and as a resident-not-in-training (ANIOS) in pediatrics and psychiatry (Zuyderland, Heerlen).

In 2011, Denise started her PhD project 'Lifestyle factors and risk of head-neck cancer subtypes: a prospective cohort study' at Maastricht University, supervised by prof. dr. ir. Piet van den Brandt, prof. dr. Bernd Kremer, and dr. Leo Schouten. She performed her research at the Department of Epidemiology, in collaboration with the Department of Otorhinolaryngology, Head & Neck Surgery (Maastricht University Medical Center+). Since 2012, Denise has also been volunteering as supervisor of a psychosocial support group for young adults with cancer at the Toon Hermans Huis Maastricht.

With her ongoing interest in mental and physical health and their relationship with lifestyle, Denise aims to pursue a career in psychotherapy and health counseling.

List of publications

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1 **Maasland DHE**, Schouten LJ, Kremer B, van den Brandt PA. Toenail selenium status and risk of subtypes of head-neck cancer: the Netherlands Cohort Study. Eur J Canc 2016;60:83-92.

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Submitted

6 Perloy A, **Maasland DHE**, van den Brandt PA, Kremer B, Schouten LJ. Intake of meat and fish and risk of head-neck cancer subtypes in the Netherlands Cohort Study.

Oral presentations

7 Body mass index and risk of subtypes of head-neck cancer: results from the Netherlands Cohort Study. 227th General Meeting of the Dutch Otorhinolaryngology Society. Nieuwegein, The Netherlands; November 2015.

8 Consumption of vegetables and fruits and risk of subtypes of head-neck cancer: results from the Netherlands Cohort Study. 223rd General Meeting of the Dutch Otorhinolaryngology Society. Maastricht, The Netherlands; November 2013.

9 Alcohol consumption, cigarette smoking and risk of subtypes of head-neck cancer: results from the Netherlands Cohort Study. 4th World Congress of the International Academy of Oral Oncology. Island of Rhodes, Greece; May 2013.

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Poster presentation

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