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Alcohol-folate interactions in women's oral cancer risk: A prospective cohort study

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Abstract

Background—The aim of this cohort study was to quantify the effect of alcohol in the risk of oral cancer in different strata of folate intake, controlling for known confounders.

Methods—A cohort of 87,621 women in the Nurses' Health Study was followed up from 1980 to 2006, and 147 incident oral cancer cases were reported and confirmed. Data on alcohol intake and diet was obtained via self-reported Food Frequency Questionnaires every 4 years. Cox Proportional Regression analysis was conducted to estimate the adjusted risk ratios (RR) and 95% confidence intervals (CI).

Results—When compared to non-drinkers, the adjusted relative risks (95% CI) for alcohol intake were 0.59 (0.39-0.87) for 0.1-14.9 g/day; 1.15 (0.67-1.97) for 15-29.9 g/day; and 1.92 (1.08-3.40) for >30 g/day. We observed a significant interaction between alcohol and folate intake (p-value = 0.02). The cancer risk for subjects with high alcohol drinking (>30 g/day) and low folate intake (<350 μg/day) was significantly elevated (RR: 3.36; 95% CI: 1.57-7.20) as compared to non-drinkers with low folate. The risk associated with high alcohol (>30 g/day) was reduced to 0.98 (0.35-2.70) in the high folate (>350 μg/day) group, as compared to non-drinkers with high folate.

Conclusions—High alcohol intake is associated with significantly increased oral cancer risk, especially in women with low folate intake.

Impact Statement—A significant interaction between alcohol and folate intake seems to affect oral cancer risk in women, a finding with potential public health utility.

Keywords

Acetaldehyde; alcohol; antagonism; cancer; cohort study; epidemiology; epithelial; female; folate; folic acid; interaction; malignancies; mouth; nurses; oral; oral cancer; tobacco; risk; women

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Introduction

Oral cancer is a global health problem with annual estimates of 300,000 new cases globally and 35,000 new cases in the US (1,2). The rates vary globally based on the prevalence of risk factors such as tobacco, alcohol, and betel quid use (3). About 90% of the cases are squamous cell carcinomas and the overall five-year survival rate for oral cancer is approximately 50% (4). The rates among women are lower when compared to men, however recent trends show an increase in the incidence among women (5). In 1950, the male to female ratio was 6:1 but by the year 2002 the gap had closed to a ratio of 2:1. Oral cancer occurs most commonly in adults aged 40 years and above. Established risk factors for oral cancer are tobacco use, excessive alcohol intake, betel quid use (a prevalent habit in South and East Asia involving intra-oral use of a mixture of areca nut, lime, and tobacco wrapped in a betel leaf), a diet poor in fruit consumption and immunosuppression (6).

Excessive alcohol consumption has been consistently associated with risk of cancer, particularly cancers of the liver, digestive tract and oral cavity (7). In the United States, men who consume 2+ drinks per day and women who consume 1+ drinks per day seem to experience higher incidence of oral cancer (8). The majority of the studies in oral cancer epidemiology involve men, especially smokers. Among non-smokers, oral and pharyngeal cancers are relatively rare and few published studies have included enough cases to provide meaningful information about the effect of alcohol especially among women (9). While some evidence indicates that women may be more susceptible than men to alcohol-induced carcinogenesis (10,11), the magnitude of alcohol's effect on the oral cancer risk of women remains under-studied.

Studies in recent years have reported an association between folate deficiency and risk of cancer (12,13). For example dietary folate intake has been found to be associated with lower risk of colorectal cancer (14,15). In oral cancer, only a few case-control studies have considered the possible role of folate, and their results have been inconsistent (16-19). Similarly inconsistent are the analyses that consider low folate and high alcohol intake (19), despite the fact that a strong interaction seems to exist in colon cancer, whereby risk is highest in those with high alcohol drinking and low folate intake (14,20).

To our knowledge our study is one of very few prospective studies of oral cancer in women examining the interaction between alcohol and folate in oral cancer risk.

Materials and Methods

Study Population: Nurses' Health Study

The study population included women who participated in the Nurses' Health Study I (NHS), an ongoing prospective cohort that was initially established in 1976 (21,22). Historically, in 1976 a total of 121,701 married registered female nurses from 11 US states between the ages 30 – 55 years responded to the baseline questionnaire on lifestyle factors, behaviors, personal characteristics, medical history and diagnosis of diseases. Since then, follow-up questionnaires have been mailed every 2 years to update information on risk factors and the onset of newly diagnosed diseases. Each follow-up cycle has achieved overall response rates of 90% or higher. This investigation was approved by the Institutional Review Board (IRB) of the Brigham and Women's Hospital, Boston, MA. Deaths are identified from the post office, next-of-kin, and the National Death Index; with an estimated ascertainment of 98% of all deaths (23). In 1980 the first semi-quantitative Food Frequency Questionnaire (FFQ) was mailed to the participants. Follow-up diet questionnaires were mailed in 1984, 1986, and every 4 years thereafter.

Exposure assessment

The baseline FFQ in 1980 included 61 questions on food and food products consumed including dairy products, fruits, vegetables, meats, sweets, baked goods, cereals, and beverages (24). Participants reported detailed information on the frequency of consumption over the past year for each food item using common units or portions. Total nutrient intakes were calculated by multiplying the frequency of consumption by the nutrient content and summing the products over all food items (25). The Harvard University Food Composition Database, which was derived from the United States Department of Agriculture (USDA) database was used as the source for calculations of nutrient values (26). The use of the semi-quantitative FFQ to obtain information on intake of dietary factors has been found to be valid and reliable in the NHS (27,28). The validity of the FFQ was compared to 1-year diet records and the mean nutrient intake reported in the FFQ was found to be within 10% of the measurements in the diet records. Information on vitamins and mineral supplements were recorded in the FFQs starting in 1980 (29). Both current intake and total years of intake were recorded. For folate, we calculated "total folate" measured in micro-grams per day (μg/d) to be the sum of folate intake from food sources, vitamin supplements, and fortification of foods with folic acid. Assessing total folate via the FFQ has been validated in the NHS cohort and has been shown to correlate with erythrocyte folate concentrations ($r = 0.55$) (25).

With regard to alcohol, detailed data was first collected in the 1980 FFQ, and was updated in all subsequent FFQs. Information on total alcohol intake; type of alcoholic drink (beer, liquor and wine) and frequency of consumption in the previous year are routinely collected. For purposes of the statistical analysis, the alcohol content of each beverage was estimated to be 13.1 grams (g) per 12-ounce bottle or can of beer, 11 g per 4-ounce glass of wine, and 14 g per standard drink or shot of liquor (30). Total alcohol intake was calculated as the sum of all alcoholic beverages. Former alcohol drinking was evaluated at baseline based on whether the participant greatly increased or greatly decreased their intake in the previous 10 years and if they were currently non-drinkers. The method of assessing alcohol using a self-administered questionnaire has been shown to be valid and reproducible in the NHS cohort (31). Alcohol consumption reported in the FFQ in 1980 and 1981 was highly correlated with the information assessed by 1-week diet records ($r = 0.90$). Four years later in 1984 the participants reported alcohol consumption in the previous four years and this was also highly correlated with the weekly diet records ($r = 0.84$).

Outcome assessment: "Oral Cancer"

The outcome of our study "oral cancer" was first self-reported in 1976 and was subsequently updated every two years thereafter. For our study, self-reports were followed by reviews of the medical and pathology reports, and were adjudicated by study physicians who were blinded to risk factor information. The International Classification of Diseases, ninth revision (ICD-9) codes141-146, and 148 were used to categorize the oral cancers according to site/region (Appendix 1) (32). To avoid including cancers with etiologies that are known to be different than that of epithelial cancer, we excluded cancers of the lip and nasopharynx (ICD 140, 147, 149). Following the above exclusions during the follow-up period from 1980-2006, a total of 147 confirmed oral cancer cases were included in our analyses.

Data Analysis—For each participant, the follow-up period starts from the date of return of baseline questionnaire (June 1980) to the date of diagnosis of oral cancer, death, or end of follow-up (December 2006) whichever occurs first. Those who did not complete the diet questionnaire in 1980, and those diagnosed with cancer in 1980 (except for non-melanoma skin cancer) were excluded, leaving 87,621 participants with complete information to participate in the analyses.

At each follow-up cycle only those who were free of oral cancer and who were alive were included. Confirmed cases were censored from subsequent follow-up.

Total alcohol intake was categorized as 0 gms/day; 0.1—14.9 gms/day; 15-29.9 gms/day; and ≥30 gms/day. Total folate intake (μg/d) was used as a continuous variable and as a categorical variable. The mean folate value in our cohort was used as the approximate cut-off level for the binary folate variable (<350 μg versus ≥350 μg). To evaluate the joint association between alcohol and folate a combination variable was created for three alcohol levels, 0-4.9 g/d, 5-14.9 g/d and ≥15 g/d, across binary folate categories. The cumulative average updating method was used for all of the nutrient variables, including alcohol and folate. The diagnosis of oral cancer was modeled in relation to the cumulative averages from all available dietary questionnaires up to the start of each 2-year follow-up interval. The cumulative average updating method is recommended to estimate long-term intakes and has been found to reduce within-person variation (33). Mechanistically, for the outcome "oral cancer" diagnosed between 1980 and 1984, alcohol data from the 1980 FFQ was used. For outcomes reported between 1984 and 1986, the average of the alcohol data from 1980 and 1984 FFQs was used. For outcomes reported between 1986 and 1990, the average of the 1980, 1984, and 1986 FFQs was used; and so forth. Once a participant was diagnosed with oral cancer, her alcohol information was not updated starting from the beginning of the time interval in which they were diagnosed.

Multivariate Cox regression analysis with time-varying covariates was performed to calculate hazard ratios and 95% confidence intervals (CI) as estimates of relative risk (RR). Linear trends were evaluated by entering a single ordinal variable, and Wald test for trend were conducted to estimate the p-value for the test for trend (34). The alcohol-folate interaction was evaluated by entering an interaction term in the multivariate model. Subsequently, Likelihood Ratio Test (LRT) was used to compare models with and without the interaction term. Also estimated were the joint effect estimates for alcohol-folate levels and stratum-specific estimates for alcohol across folate categories. Multivariate models were adjusted for age (years), follow-up time (2 years), smoking status (current versus past/never), pack-years of smoking (0, 1-19 py, 20-39 py, ≥40 py), and total folate intake (<350 μg versus ≥350 μg).

Results

Participants' characteristics at baseline are shown in Table 1. Among the 87,621 participants, 32% reported no alcohol intake in 1980 and 12% reported ≥15 g/day of alcohol intake. The overall mean age among the participants at baseline was 47 years. Those who were never smokers were more likely to be non-drinkers or light drinkers. Those who consumed ≥30 g/day of alcohol also were more likely to report current cigarette smoking. The mean folate intake in this population was 353 μg/day. Approximately 64% of the participants reported <350 μg/day of folate intake across the alcohol intake categories.

As mentioned previously, we identified a total of 147 confirmed cases of oral cancer during the follow-up period 1980-2006. The distribution of cases by level of alcohol intake is shown in table 2. In the age-adjusted analysis, cumulative average alcohol intake was significantly associated with oral cancer among women who consumed ≥30 g/day when compared to those who do not drink (RR: 2.70; 95% CI: 1.57-4.65). Low to moderate alcohol drinking was associated with lower risk (RR: 0.57; 95% CI: 0.39-0.84). In the multivariate model adjusting for age, follow-up time, pack-years of smoking, smoking status and folate intake, high alcohol intake of ≥30 g/day was significantly associated with increased risk (RR: 1.92; 95% CI: 1.08-3.40) compared to non-drinkers. As expected, the risk increased further for subjects who were heavy drinkers and current smokers. We also considered former alcohol drinking, the type of alcoholic beverage consumed, fruit intake, multi-vitamin use and marital status as possible confounders, but these variables were not significantly associated with risk and were

later dropped from consideration. The risk for those who consumed 15-29.9 g/day of alcohol was not significantly higher when compared to non-drinkers. In the multivariate analysis, folate intake of ≥350 μg/day was inversely associated with oral cancer risk, (approximately 20%), however the result was not significant (results not shown).

As mentioned earlier, the interaction between alcohol intake and folate was evaluated by including an interaction term in the multivariate model and evaluating its significance using the Wald statistic. The interaction test was statistically significant, with a p-value of 0.02, when controlling for smoking, age, and follow-up time. We also assessed the interaction using the Likelihood Ratio Test method (LRT) comparing the nested model without the interaction with the one that included the interaction term; the LRT was found to be significant (p = 0.02). The effect of alcohol intake on the risk for oral cancer for each stratum of folate intake i.e., <350 μg/d and ≥350 μg/d was evaluated, adjusting for pack-years, smoking status, age and follow-up time (Table 3). Among those with ≥350 μg/d of folate intake, the risk was lower for all categories of alcohol drinking when compared to non-drinkers, though the overall trend was not significant. Among those with low folate intake (<350 μg/d), high alcohol drinkers (≥30 g/d) had significantly higher risk (RR: 3.36: 95% CI: 1.57-7.20) compared to non-drinkers.

Discussion

One of the major findings of this prospective analysis of 87,621 women with 26 years of follow-up was that high alcohol intake (≥30 g/day or approximately 2 drinks/day) seems significantly associated with increased risk of oral cancer after adjusting for age, tobacco use, folate intake, and follow-up time. Our results also show a significant interaction between alcohol consumption and folate intake; alcohol seems to increase the risk significantly only for those with low folate intake (<350 μg/d).

The World Health Organization (WHO) Global Status Report (7) places the relative risks for oral cancer among females at 1.45 for those consuming 0-19.99 g/d of alcohol, at 1.85 for those consuming 20-39.99 g/d of alcohol and at 5.39 for those consuming 40+ g/d of alcohol. While it is helpful to have such summary estimates, a qualitative assessment of the published literature indicates that studies on the alcohol-oral cancer risk association are mostly case-control studies that involve men, predominantly smokers (3,11). In men, prospective studies agree with case-control studies in that high levels of alcohol intake lead to higher cancer risk (35,36). A large multi-center pooled analysis of 15 studies from North America, Europe and South America showed that overall, for men and women, the odds ratio for ≥3 drinks/day versus no drinking was 2.04 (95% CI = 1.29 to 3.21), with a weak effect of alcohol among never smokers (37). But specifically among women, the role and extent of alcohol as a risk factor of oral cancer has been inconsistent (37-40), possibly because the studies have been mostly case-control in design and have very few women participants. In a well-controlled large study of 1,114 patients and 1,268 population-based controls, Blot et al showed that alcohol was an independent risk factor for oral and pharyngeal cancer, with the risk estimates been similar among men and women (38). A case-control study in Italy found that the risk increased among non-smoking females only for those consuming ≥35 drinks per week, a heavy consumption equivalent to 5 drinks per day (39). In this group of heavy drinkers, women experienced higher risk (OR=14.4; 95% C.I: 4 2.2–92.7) than men (OR=2.4; 95% C.I: 4 0.5–12.1), but according to the authors the risk estimates were not statistically heterogeneous. In a similar case control study of oral cancer in Greece, Zavras et al. confirmed a higher risk of oral cancer for men who were exposed to high amounts of alcohol, but could not confirm increased risk for alcohol in women (40). A possible explanation for the gender risk differences was that very few women in Greece consume high quantities of alcohol regularly. Macfarlane et al. conducted a combined analysis of data from three large case-control studies of oral cancer conducted in the United States, Italy and China. They found that while risks associated with tobacco smoking were generally

consistent across centers, those for alcohol were not (41). In France, alarmed by the fact that oral cancer incidence among women increases annually, Girod et al. studied the characteristics of 171 women with oral cancer who sought care in a hospital ward. Interestingly, the authors report that 51.5% of the cases did not use tobacco and 65.5% did not drink alcohol. Eighty two women with cancer reported no tobacco and no alcohol drinking. Of the women who reported alcohol drinking, the majority consumed more than 100 g of alcohol per day (42). Questions remain about the etiology of cancer in this low risk group and ascertainment bias of the alcohol self-reports cannot be excluded.

More recently (2009), Weikert et al. studied lifetime and baseline alcohol intake within the European Prospective Investigation into Cancer and Nutrition (EPIC), a prospective study of 271,253 participants across Europe. The authors reported 6-fold elevated risks for women consuming >30g/d of lifetime exposure as compared with women who consumed 0.1-6.0 g/day alcohol intake at lifetime. Compared to men, the effect of lifetime alcohol consumption was stronger among women ($p_{interaction}$ =0.045); for oral cavity cancers, the relative risk per 10g/increase was 1.09 for men and 1.26 for women (95% C.I: 1.07-1.49). This longitudinal study supports a higher predisposition of women to alcohol-related carcinogenesis (43).

Multiple mechanisms are involved in alcohol-induced carcinogenesis and several studies have been conducted in both humans and animals (44). Locally in the oral cavity the ethanol in alcohol produces epithelial atrophy which increases the penetration of carcinogens through the oral mucosa (45). This penetration may occur either due to the elimination of the lipid component of the barrier present in the epithelial layer or due to reorganization of the cell membrane by ethanol which increases permeability. Ethanol by itself has not been proven to be carcinogenic (46); however, acetaldehyde, the primary metabolic product of ethanol, has been established as carcinogenic in animal experiments and possibly carcinogenic in humans as well (47). Acetaldehyde causes DNA mutations and interferes with DNA synthesis and repair (48). Acetaldehyde can also be produced by oral bacteria, adding to the total circulating salivary acetaldehyde. With regard to the genetics of alcohol metabolism, the production of acetaldehyde from ethanol is facilitated by alcohol dehydrogenase (ADH). Acetaldehyde is further converted to acetate by aldehyde dehydrogenase (ALDH). It is interesting to note that genetic polymorphisms of both ADH and ALDH are associated with increased risk of head and neck cancers (49).

Our adjusted analysis found that high alcohol intake (≥30 g/day) was associated with significantly increased risk for oral cancer as compared to non-drinking (Table 2), a finding consistent with the published literature. We also evaluated the interaction between smoking and alcohol (results not shown) and confirmed that the risk is highest for those exposed to high intakes of both tobacco and alcohol. Again this result is consistent with prior reports (50) and provides external validity to our results. However, low or moderate alcohol intake (0.1-14.9 g/day) was not associated with an increased risk for oral cancer; rather, it was associated with decreased risk. The literature on the role of low or moderate consumption on the risk to develop oral cancer or on a possible threshold effect is inconsistent. Some case-control studies have observed a u-shaped relationship between alcohol consumption and the risk of oral cancer. More specifically, a u-shaped relationship has been reported in 6 out of 19 case-control studies; according to these studies, drinking small amounts of alcohol seems to be protective but drinking high amounts seems to be detrimental (11). In other studies, the existence of a u-shaped curve could not be assessed because of using <4 drinks/day as the lowest category cut-off point. This u-shaped relationship has been observed mostly in men, possibly because of the low samples of women in the highest consumption strata (51). In our study, the apparent u-shaped association with alcohol was observed only among women with high folate.

It is currently unclear why alcohol at lower levels may be protective against oral cancer. It is plausible that low levels of alcohol in post-menopausal women are correlated with increased insulin sensitivity and elevated estrogen levels (52). Recently, an analysis of 74,372 women participants of the NIH-AARP cohort showed a protective effect of menopausal hormone therapy (MHT). Women who had especially received estrogen-progestin MHT had a 0.47 times the risk to develop squamous cell carcinoma of the upper aerodigestive tract (protective association) (53). However this finding needs replication.

Studies conducted so far on the role of folate in oral or head and neck cancer have been case-control and cross-sectional in design. While low serum folate levels have been observed in head and neck cancer patients before (54,55) the analytic studies report mixed results (17,19, 56). Folate was associated with slightly increased risk of oral cavity cancers (OR=1.3; 95% C.I: 0.8-2.2) in a hospital-based case control study conducted in Uruguay, but the results were not statistically significant (17). A hospital based case-control study from Italy and French-speaking Switzerland that enrolled 749 patients and 1772 controls (115 were female cases) reported an inverse association with folate intake (OR: 0.53; 95% CI: 0.40–0.69) for the highest tertile of intake (≥301) (18). In that study the combined effect of high alcohol intake (≥38 drinks/week) and low folate intake (<235.9 μg/day) showed a 22-fold increase in risk when compared to those with low alcohol (<14.5 drinks/week) and high folate intake (>300.7 μg/day). While the overall direction of the results agrees with our findings, such disproportionately strong effects may be due to higher levels of exposure (much higher alcohol intake and significantly lower folate among hospital cases) as compared with our study population of women nurses in the United States. Significantly decreased levels of folate were also observed in a small case control study of 50 subjects with oral leukoplakia, a clinical condition often associated with dysplasia and oral cancer (57). In contrast, a population-based case control study of oral cancer in Puerto Rico concluded that oral cancer risk is not related to folate (19). Besides the conclusion, the authors noted that increased folate intake was associated with decreased oral cancer risk, with an adjusted odds ratio in highest vs. lowest quartile of 0.6 (95% CI: 0.4, 1.0), but this protection was restricted to folate intake from fruit (adjusted OR = 0.4, 95% CI: 0.2, 0.6; p(trend) = 0.0001), and not from other dietary folate sources.

The joint association of alcohol and folate on the risk of major chronic disease has been studied previously in the NHS and a significant interaction between alcohol and folate intake has been reported in breast and colon cancers and alcohol-related cancers as a group (25).

The mechanism whereby low folate accentuates the association between alcohol and oral cancer risk is currently unclear. Folate is essential for DNA synthesis and repair, and for the methylation of biological substances, including phospholipids, DNA, and neurotransmitters (58). DNA methylation is important in the maintenance of DNA stability and in gene expression. Alcohol interferes with several aspects of normal folate transport and metabolism including dietary folate intake, intestinal absorption, transport to tissues, folate storage in the liver and urinary excretion(59-61). Human and animal experiments suggest that the acute toxicity of alcohol on folate is primarily related to its influence on folate metabolism in the liver (58). Because the metabolism of acetaldehyde from ethanol also occurs locally in the oral cavity, cleavage of folate can occur in the micro-environment of the oral cavity, deterring the positive effects of folate (62). Thus, by impairing folate status and metabolism, ethanol may enhance cancer risk both systematically and locally and disrupt the DNA synthesis, repair and methylation of the squamous epithelial oral cell (48).

With regard to the gender differences, it is currently unclear if there are differences in the effect of alcohol intake on oral cancer. The present study has enrolled and followed up only women, thus precluding the possibility of any direct comparisons. Most studies have involved men, with women being grossly under-represented among study participants. It is possible that there

may be differences in susceptibility to alcohol-induced carcinogenesis, with women being more susceptible, although the evidence is inconclusive (10,11). It is also possible that a gender effect may exist in alcohol gastric metabolism, which in turn may affect the bio-availability of acetaldehyde in the oral epithelial tissue. In men, the blood level of alcohol is higher when given intravenously as compared with drinking (63). However, women have been found to have similar blood levels of alcohol irrespective of whether the alcohol was given orally or intravenously. This gender difference could be due to lower gastric metabolism of ethanol when compared to men (10,11). Studies on cancer of the liver have shown that liver damage is higher among females when compared to men at lower intake of alcohol and shorter duration of intake. Hormonal differences in men and women can also play a role in the joint effect of folate and alcohol and how they influence differences in oral cancer risk (52).

While we were able to assess the main question of interest, the effect of alcohol consumption in oral cancer risk stratifying for levels of folate intake, there are power limitations in studying interactions with other covariates due to low number of cases in some strata. Despite the various ways that the analysis controlled for smoking, residual confounding (by smoking) is always possible, especially among the low-folate intake group. As with all epidemiologic studies using a self-administered diet questionnaire, the possibility of measurement error cannot be completely eliminated. However, in our study we minimized the bias from measurement error by using cumulative averages of repeated measurements over a long follow-up (28,29,31). Further, the main exposure of interest, alcohol intake measurements as reported in the FFQ, have been studied extensively and have been validated in the past (31). Misclassification if any will be non-differential thus leading to underestimation of the true association. The potential for misclassification of oral cancer is very minimal as the cancer diagnosis was confirmed by blinded study physicians. With regard to how representative the study subjects are of the US population of women, there are some concerns with regard to generalizability as the participants are predominantly White. While the range of risk factors could be slightly different from US women in general, we expect the biologic relations to be similar. Strengths of our study include a large prospective cohort study design, and a follow-up of 26 years with updated information on several time-varying confounders. The NHS cohort included married registered nurses at baseline. Therefore the cohort is homogenous with regard to SES, level of education, and health awareness, reducing possible confounding by these factors. Misclassification of alcohol and nutrients is minimal in our study as we used questionnaires that have been found to be valid and reliable (31). Additionally, the NHS cohort includes a group of health professionals that are dedicated to health sciences (by means of consistent volunteering in the Study for over 26 years) and so we assume that there will be a good degree of accuracy in reporting health information.

In conclusion, alcohol intakes of >2 drinks per day leads to 2-fold increase in the risk of oral cancer in women compared to non-drinkers. This risk increases further in drinkers with low intake of folate. Women with high folate intake have lower alcohol-related risk for oral cancer. Along with discontinuation of tobacco use, public health recommendations to reduce the incidence of oral cancer in women must include minimizing alcohol consumption to a level of 0-1 drinks per day, and the increase of folate intake.

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Appendix 1. Classification of diagnostic codes for oral cancer by site/region according to the International Classification of Diseases, ninth revision (ICD-9)

ICD-9 codes	Definition
140	Malignant neoplasm of lip
141	Malignant neoplasm of tongue
142	Malignant neoplasm of major salivary glands
143	Malignant neoplasm of gum
144	Malignant neoplasm of floor of mouth
145	Malignant neoplasm of other and unspecified parts of mouth
146	Malignant neoplasm of oropharynx
147	Malignant neoplasm of nasopharynx
148	Malignant neoplasm of hypopharynx
149	Malignant neoplasm of other and ill-defined sites within the lip

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Table 1

Baseline Characteristics of Nurses' Health Study I (NHS) participants by alcohol intake in 1980: N = 87,621

	Baseline alcohol intake in grams per day (g/d)			
	0 g/d	**0.1-14.9 g/d**	**15-29.9 g/d**	**30+ g/d**
N (%)	28221 (32.2)	48891 (55.8)	6062 (6.9)	4447 (5.1)
Age (mean, years)	46.9	46.3	47.2	48.2
Amount of Alcohol intake (mean, g/d)	0.0	5.0	20.9	41.6
Reported reduction in alcohol at baseline (%)	10.3	10.4	8.2	8.3
Folate (μg/d)				
Total folate (μg/d, mean)	353.7	351.5	364.6	358.3
<350 μg/d (%)	65.1	64.7	62.2	63.4
≥350 μg/d (%)	34.9	35.3	37.8	36.6
BMI (kg/m^2, mean)	25.4	24.1	23.3	23.6
Smoking status† (%)				
Never	57.7	40.6	27.2	18.6
Past	19.9	30.0	38.0	27.3
Current	22.4	29.5	34.9	54.1
Pack-years of smoking† (%)				
Mean pack-years	9.0	11.4	15.1	23.9
1-19 pack-years	22.3	34.8	39.6	28.5
20-39 pack-years	14.3	18.5	24.2	30.9
40+ pack-years	5.7	6.2	9.1	22.0
Total fruit intake (servings/day, mean)	2.2	2.1	2.0	1.6
Total calories (calories/day, mean)	1564.2	1543.2	1626.8	1756.1
Multi-vitamin user (%)	32.8	34.2	36.5	35.4
Married (%)	92.3	91.1	90.0	88.4
Race (%)				
White	82.8	86.1	87.4	83.3
Black	1.3	1.0	0.5	0.5
Hispanic	0.4	0.4	0.3	0.1
Asian	1.2	0.4	0.2	0.2
Other	14.2	12.1	11.7	15.9

†The smoking categories do not add up to the total due to missing values

Table 2

Age-adjusted and multivariate risk ratios (RR) and 95% confidence intervals (CI) for oral cancer among women by level of alcohol intake (g/d)

	Risk ratios (95% CIs) by level of alcohol intake[§]			
	0 g/d	**0.1-14.9 g/d**	**15-29.9 g/d**	**30+ g/d**
Cases	43	64	21	19
Age-adjusted[*]				
RR (95% CI)	1.00	**0.57 (0.39-0.84)**[†]	1.29 (0.76-2.18)	**2.70 (1.57-4.65)**[‡]
Multivariate-adjusted[**]				
RR (95% CI)	1.00	**0.59 (0.39-0.87)**[†]	1.15 (0.67-1.97)	**1.92 (1.08-3.40)**[†]

[*] Adjusted for age and follow-up time

[**] Adjusted for age, follow-up time, pack-years of smoking, current smoking status and folate intake

[†] p-value ≤0.01

[‡] p-value ≤ 0.001

[§] One alcoholic drink contains approximately 11-14 grams of alcohol: Regular beer = 12.8 g, light beer = 11.3 g, wine = 11 g, and liquor = 14 g

[¥] Note: Significant p-value for alcohol-folate interaction (p=0.02) in multivariate model controlling for age, pack-years of smoking and current smoking status (not shown in table)

Table 3

Stratum-specific estimates for total alcohol intake across levels of folate intake among women with oral cancer

	Stratum-specific RR(95% CI)*			
	Folate ≥ 350 μg/day		Folate <350 μg/day	
Alcohol Intake§	Cases (n = 63)	RR (95% CI)	Cases (n = 84)	RR (95% CI)
0 g/d	27	1.00	16	1.00
0.1 - 14.9 g/d	21	**0.29 (0.16-0.51)**†	43	1.11 (0.61-1.99)
15 - 29.9 g/d	10	0.90 (0.42-1.94)	11	1.53 (0.69-3.38)
30+ g/d	5	0.98 (0.35-2.70)	14	**3.36 (1.57-7.20)**‡

*Adjusted for age, follow-up time, pack-years of smoking and current smoking status

†p-value<0.0001

‡p-value<0.01

§One alcoholic drink contains approximately 11-14 grams of alcohol: Regular beer = 12.8 g, light beer = 11.3, wine = 11 g, and liquor=14 g