Fruit and vegetable consumption and squamous cell carcinoma of the esophagus in Japan: The JPHC study

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Epidemiological studies have consistently demonstrated a decrease in the risk of esophageal squamous cell carcinoma (SCC) associated with higher fruit and vegetable intake, although the association has been examined predominantly in case-control studies. Here, we conducted a population-based prospective cohort study among middle-aged Japanese men. Lifestyle characteristics were investigated using a self-administered questionnaire, which included a validated food frequency questionnaire with 138 food and beverage items. We followed a total of 38,790 men aged 45–74 years. A Cox proportional hazards model was used to estimate the hazard ratios (HRs) and 95% confidence intervals (95% CIs) for esophageal SCC, with adjustment for potential confounders. During 297,651 person-years of follow-up, a total of 116 men were newly diagnosed with esophageal SCC. An increase in consumption of total fruit and vegetables by 100 grams per day (g/day) was associated with an 11% decrease in the incidence of esophageal SCC (95% CI: 1–21%). In particular, a higher intake of cruciferous vegetables was associated with a significant decrease in risk (HR per 100 g/day: 0.44; 95% CI: 0.23–0.82). Stratified analyses revealed that the beneficial effect of fruits and vegetables was not able to completely offset the harmful effect of tobacco and alcohol. Our findings provide further evidence for the protective role of fruits and vegetables in the development of esophageal SCC. To effectively reduce the burden of this disease, however, strong efforts should be placed on smoking and drinking cessation. Finally, cruciferous vegetables deserve further investigation as foods possibly effective in the prevention of esophageal SCC.

Key words: esophageal cancer; fruits; vegetables; cruciferous vegetables; prospective study

Squamous cell carcinoma (SCC) of the esophagus occurs at a high frequency in many developing countries. In Asia, areas with an extremely high incidence of the disease cluster around the region called the “esophageal cancer belt”, which stretches from northern Iran to north-central China. Because the prevalence of tobacco smoking and alcohol drinking in this region is not markedly high, attention has focused on the role of indigenous dietary habits, particularly the tendency toward lower consumption of fruits and vegetables.

Observational studies have consistently demonstrated a decrease in the risk of esophageal SCC associated with a higher intake of fruits and vegetables combined, albeit predominantly in case-control studies, which are prone to several biases. To date, only one cohort study has evaluated the association.

Various groups of fruits and vegetables have been investigated separately to identify diets effective in cancer prevention. Among the results, cruciferous vegetables have attracted considerable attention, with experimental studies showing the possibility that particular ingredients in cruciferous vegetables, such as isothiocyanates, inhibit carcinogenesis. To our knowledge, however, only three epidemiological studies have evaluated the association with esophageal SCC.

Here, to provide further evidence on the association of combined fruit and vegetable intake with SCC of the esophagus, we analyzed data from a population-based prospective cohort study among middle-aged Japanese men. We also investigated fruit and vegetable subgroups, with particular interest in cruciferous vegetables, whose consumption in the Japanese population is relatively high.

Material and methods

Study population

The Japan Public Health Center-based Prospective Study (the JPHC Study) consists of two cohorts, with Cohort I launched in 1990 and Cohort II in 1993. Details of the study design have been described elsewhere. The study protocol was approved by the institutional review board of the National Cancer Center, Tokyo, Japan.

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The study population was defined as all registered Japanese inhabitants in 11 public health center (PHC) areas, aged 40–59 years in Cohort I and 40–69 years in Cohort II. Study subjects were identified using population registries maintained by the local municipalities. In the present analysis, we excluded two PHC areas (Tokyo and Osaka), where different definitions of the study population were adopted. In addition, we did not include female subjects because the expected number of female patients with esophageal cancer was too small to adequately address sex-specific differences in risk. Initially, 57,579 men were ascertained to be eligible.

Surveys on lifestyle characteristics and medical histories of the study subjects were conducted twice. The second survey was carried out 5 years after the first, in 1995 for Cohort I and 1998 for Cohort II. A self-administered questionnaire was distributed to all study participants available at that time, not limited to the participants of the first survey. In the current analysis, we employed the data from the second survey, primarily because more comprehensive information on food intake was collected in the second survey than in the first. A total of 5,712 men were already ineligible before the second survey, consisting of 2,146 who had died, 3,433 who had moved out of their original PHC area, and 133 who had been lost to follow-up. Of the remaining 51,867 men, a total of 42,759 responded to the self-administered questionnaire of the second survey, yielding a response rate of 82.4%.

**Self-administered questionnaire**

The self-administered questionnaire of the second survey included a food frequency questionnaire (FFQ) of 138 food and beverage items with standard portions/units and nine frequency categories (for food items: almost never, once to 3 times per month, once or twice per week, 3 or 4 times per week, 5 or 6 times per week, once per day, twice or 3 times per day, 4–6 times per day and 7 or more times per day; for beverage items: almost never, once or twice per week, 3 or 4 times per week, 5 or 6 times per week, 1 glass per day, 2 or 3 glasses per day, 4–6 glasses per day, 7–9 glasses per day and 10 glasses or more per day). Standard portion sizes were specified for respective food items, with the three amount choices of small (50% smaller than the standard), medium (same as the standard) and large (50% larger). Fruit and vegetable consumption was also calculated from the responses, and expressed as grams per day (g/day), with reference to the Standard Tables of Food Composition in Japan, 5th Revised Edition.15

The FFQ asked about the average consumption of 16 fruit and 30 vegetable items in the past year. These individual items were categorized into three main groups of total fruit and vegetables (all fruit and vegetable items), total fruits (all fruit items) and total vegetables (all vegetable items); and into the five subgroups of pickled vegetables (pickled Chinese radishes, pickled green leafy vegetables, pickled plums, pickled Chinese cabbage, pickled cucumbers and pickled eggplant), cruciferous vegetables (cabbage, Chinese radishes, komatsuna, broccoli, Chinese cabbage, leaf mustard and (Swiss) chard), green leafy vegetables (spinach, sweet pepper, Chinese chives, garland chrysanthemums, chingen-sai and mugwort), yellow vegetables (carrots, tomatoes, pumpkins and tomato juice) and citrus fruits (mandarin oranges, other oranges and 100% orange juice).

We examined the validity of the FFQ using 14- or 28-day dietary records as the gold standard.16,17 showing Spearman’s correlation coefficients of 0.57 for fruits and of 0.38 for vegetables.18 Reproducibility of the FFQ was also assessed by administering two questionnaires 1 year apart, showing Spearman’s correlation coefficients of 0.57 for fruits and of 0.59 for vegetables.18 Fruit and vegetable consumption were moderately correlated with each other (Spearman’s correlation coefficient: 0.37).

The FFQ also inquired about the frequency of alcohol drinking and the number of the standard units consumed per occasion for five different alcoholic beverages (sake, shochu/awamori, beer, whisky and wine). We have detailed elsewhere the method by which ethanol intake is calculated from the FFQ as well as its validity and reproducibility.20 The study subjects were divided into two drinking groups in relation to their frequency: occasional drinkers (<1 occasion per week) and regular drinkers (≥1 occasion per week). Regular drinkers were further stratified into four categories in proportion to their ethanol consumption (<150, 151–300, 301–450 and >450 g per week).

The self-administered questionnaire of the second survey also contained questions about smoking habits. The cohort participants were first asked about their smoking status (current, past and never). For current smokers, exposure was expressed as cigarettes per day in three categories (<20, 20–29 and ≥30 cigarettes per day). Further, quitting periods of past smokers were defined based on smoking status at the first survey and divided into three categories (5 years or less, more than 5 years and uncertain).

Among male respondents to the questionnaire of the second survey, 37 had suffered from esophageal cancer before the survey and were excluded. A further 3,932 men were excluded due to extreme total energy intake (<800 or >4,000 kcal per day) or missing information on alcohol drinking or tobacco smoking, leaving 38,790 men for analysis.

**Follow-up**

Subjects in Cohort I were followed from 1995, and subjects in Cohort II from 1998, until December 31, 2004. Changes in residence status, including survival, were identified annually through the residential registry in each study area or, for those who had moved out of the original study area, through the municipal office in the area to which they had moved. Mortality data for people in the residential registry are forwarded to the Ministry of Health, Labour and Welfare and coded for inclusion in the National Vital Statistics. Residency and death registration are required by the Basic Residential Register Law and Family Registry Law, respectively, and the registries are believed to be complete. Here, information on the cause of death was based on death certificates from the respective PHC for those who had stayed in the original study area. During the follow-up period in this study, 3,096 men died (7.9%), 1,120 moved out of the original area (2.9%) and 12 were lost to follow-up (0.03%).

The occurrence of cancer was identified by active patient notification from major local hospitals in the study area and data linkage with population-based cancer registries, with permission from the local governments responsible for the registries. The site and histology of each cancer were coded according to the International Classification of Diseases for Oncology, 3rd Edition (ICD-O-3).21 Death certificate information was used as a supplementary information source. In the cancer registry system available for this study, the proportion of patients with incident cancers who first came to the attention of the cancer registry via a death certificate (death certificate notification, or DCN) was 11.4%, and the proportion of case patients for whom information regarding cancer diagnosis was available from a death certificate only (DCO) was 4.7%.

Person-years of follow-up were calculated for each man from the date of the second survey to the date of diagnosis of esophageal cancer, emigration from the original area or death, whichever came first; or if none of these occurred, follow-up was through to the end of the study period (December 31, 2004). Men who were lost to follow-up were censored at the last confirmed date of presence in the study area.

**Statistical analysis**

Outcome was defined as esophageal SCC newly diagnosed during the study period. We used the residual method for energy-adjustment of fruit and vegetable intake.22

A Cox proportional hazards model was applied to estimate the hazard ratios (HRs) and 95% confidence intervals (95% CIs) for SCC of the esophagus according to tertiles of energy-adjusted fruit and vegetable consumption, with the lowest tertile as the reference, while adjusting for potential confounding variables. We con-
TABLE I – BASELINE CHARACTERISTICS ACCORDING TO TERTILES OF TOTAL FRUIT AND VEGETABLE INTAKE

| Tertiles of total fruit and vegetable intake | Lowest | Middle | Highest | p-Value
|-------------------------------------------|--------|--------|---------|---------|
| Number                                   | 12,930 | 12,930 | 12,930 |         |
| Intake (g/day)                            | 170(120–209) | 322(282–362) | 544(466–672) | <0.0001 |
| Age (years)                              | 55.5(7.5) | 56.7(7.7) | 58.6(7.8) |         |
| Regular drinkers (%)                     | 76.3 | 67.9 | 54.0 | <0.0001 |
| Alcohol consumption (g/week)             | 322(161–504) | 252(127–403) | 161(81–322) | <0.0001 |
| Current smokers (%)                      | 56.4 | 46.8 | 37.0 | <0.0001 |
| Tobacco smoking (cigarettes/day)         | 20(17–30) | 20(15–30) | 20(15–25) | <0.0001 |

1Adjusted for age and PHC area. 2Further adjusted for cigarette smoking and alcohol drinking.

ducted the first analysis with adjustment for 5-year age categories at the second survey (45–49, 50–54, 55–59, 60–64, 65–69 and 70 years old or more) and nine PHC areas. In the second analysis, we further adjusted for smoking status (never, quitting period uncertain, quitting period >5 years, quitting period ≤5 years, <20, 20–29 and ≥30 cigarettes per day) and drinking status (occasional, ≤150, 151–300, 301–450 and ≥450 g per week). Linear trends in the risk of esophageal SCC were assessed by assigning ordinal values to categorical variables of fruit and vegetable intake. Furthermore, we calculated HRs and 95% CIs for SCC of the esophagus by an increase in fruit and vegetable consumption of 100 g/day. Finally, we conducted stratified analyses by tobacco smoking and alcohol drinking, the major risk factors for esophageal SCC.

Two-sided p-values less than 0.05 were regarded as statistically significant. All statistical analyses were carried out using Statistical Analysis System (SAS), Version 9.1 (SAS Institute, Cary, NC).

Results

During 297,651 person–years of follow-up (average follow-up period: 7.7 years) in 38,790 male subjects, a total of 141 men were newly diagnosed with cancer of the esophagus. Among cases, 116 were SCC (82%), 7 were adenocarcinoma (5%) and 18 were histologically undetermined (13%). We included subjects with esophageal SCC in the present analysis.

The amount of total fruit and vegetables consumed ranged from a median value of 170 g/day in the lowest tertile to 544 g/day in the highest tertile. In the study population, approximately 50% of total fruit and vegetable intake was derived from intake of eight fruit and vegetable items (carrots, Chinese radishes, onions, mandarin oranges, apples, 100% orange juice, 100% apple juice and tomato juice).

Characteristics of the study subjects varied according to the level of total fruit and vegetable consumption (Table I). Men in the higher categories of total fruit and vegetable intake were more likely to be older. The frequency of regular drinkers and the amount of alcohol consumed per week decreased with increased total intake of fruits and vegetables. Furthermore, male subjects with a higher total consumption of fruits and vegetables were less likely to be current smokers.

The age- and area-adjusted HRs of esophageal SCC for the highest compared to the lowest tertile were significantly below unity for total fruit and vegetables, total fruits and total vegetables (Table II). In addition, significant trends across tertiles were observed for all three fruit and vegetable groups. Further adjustment for smoking and drinking status attenuated the inverse associations. A significant trend across tertiles was evident only for total fruit and vegetables. Compared to those with the lowest intake, those with the highest intake of total fruit and vegetables were at a reduced risk of esophageal SCC (HR = 0.52; 95% CI: 0.30–0.88). Analyses on a continuous scale revealed that an increase in total fruit and vegetable consumption by 100 g/day was associated with a decrease in the incidence of esophageal SCC by 11% (95% CI: 1–21%). In addition to fruit and vegetable groups, we examined all plant food groups available from the FFQ (cereals, potatoes, pulses and fungi); however, no association was observed (data not shown). The individual items included in each plant food group are shown in Appendix.

To further investigate the protective effect of fruits and vegetables, we analyzed five fruit and vegetable subgroups (Table III). Of the five subgroups examined, the age- and area-adjusted HRs of esophageal SCC for the highest compared to the lowest tertile were significantly lower than unity for cruciferous vegetables.
green leafy vegetables and citrus fruits. A significant trend across tertiles was also observed for these three subgroups. On further adjustment for smoking and drinking status, a significant trend remained only for cruciferous vegetables. Analyses on a continuous scale showed that an increase in cruciferous vegetable intake by 100 g/day was related to a 56% (95% CI: 18–77%) decrease in occurrence of esophageal SCC. Consumption of cruciferous vegetables was positively correlated with that of the other fruit and vegetable subgroups, particularly green leafy and yellow vegetables. The significant decline in the incidence of esophageal SCC with cruciferous vegetables remained after additional adjustment for the remaining fruits and vegetables (HR per 100 g/day: 0.48; 95% CI: 0.25–0.93; data not shown). Finally, 46 fruit and vegetable items were individually investigated. We did not identify any specific items which made a disproportionate contribution to the observed effect of fruit and vegetable groups or subgroups (data not shown).

Presented in Table IV are HRs and 95% CIs for total fruit and vegetable intake by drinking and smoking habits. Among men consuming similar amount of fruits and vegetables, drinking of >150 g of ethanol per week and current smoking were associated with an increased incidence of esophageal SCC. In particular, men with both habits had the highest risk. On the other hand, the protective effects of total fruit and vegetables were observed for each stratum of drinking and smoking habits, but were greater in men who drank >150 g of ethanol per week and current smokers. Further, the most pronounced decrease in risk was observed among men at the highest risk (HR per 100 g/day: 0.79; 95% CI: 0.67–0.94).

We reanalyzed the data using two types of model with modification of the original model used earlier. First, daily energy intake was added to the original model. Second, crude intake of fruits and vegetables and daily energy intake were substituted for...
energy-adjusted intake of fruits and vegetables in the original model. Results were similar to those obtained with the original model (data not shown). As the symptoms of esophageal cancer might affect eating habits even before cancer diagnosis, we repeated the above analyses after excluding cases occurring in the first year. Results were essentially the same as those above.

Discussion

In this study, we identified a dose-dependent decrease in the risk of esophageal SCC associated with a higher intake of total fruit and vegetables. In particular, analyses of fruit and vegetable subgroups showed a significant decline in risk for increased intake of cruciferous vegetables. To our knowledge, this is the first prospective cohort study in an Asian population reporting that fruit and vegetable consumption collectively afford protection against the development of esophageal SCC. Our findings are consistent with those of all six previous epidemiologic studies examining the risk of esophageal SCC and total fruit and vegetable intake.

Of the five fruit and vegetable subgroups investigated in our study, only cruciferous vegetables were associated with a significant decrease in the risk of esophageal SCC after further adjustment for alcohol and tobacco use. Cruciferous vegetables have received considerable attention as foods possibly effective in cancer prevention. Experimental studies have reported the possibility that particular ingredients in cruciferous vegetables, such as isothiocyanates, inhibit carcinogenesis. However, a nonsignificant inverse association between cruciferous vegetables and SCC of the esophagus was reported in three previous epidemiologic studies. Further studies are required to replicate our results, especially in populations that consume a wide range of cruciferous vegetables.

Of particular interest were the findings of our stratified analyses by smoking and drinking habits. There was some indication that a higher intake of fruits and vegetables may afford a greater degree of protection against the occurrence of esophageal SCC among current smokers and heavy drinkers. However, the beneficial effect of fruits and vegetables was not able to completely offset the harmful effect of tobacco and alcohol. On this basis, smoking and drinking cessation should be the primary strategy to prevent this disease, especially among populations with higher prevalences of current smokers and heavy drinkers.

Our study had several associated strengths, including its population-based design. According to the National Nutrition Survey in Japan, the proportion of current smokers and regular drinkers among middle-aged men is approximately 50% and 60%, respectively. Prevalence of the major risk factors for esophageal SCC in the study population was reasonably similar to that in the general population, which facilitates the extrapolation of the present results to middle-aged Japanese men. In addition, information on fruit and vegetable intake was collected before any subsequent diagnosis of esophageal SCC. This prospective design thereby minimized the possibility of recall bias, which can undermine case-control studies. Further, response rates were relatively high, while the proportion of loss to follow-up was low.

Several limitations of the study also warrant mention. First, it remains possible that some or all of the observed associations of fruits and vegetables resulted from residual confounding by alcohol drinking and tobacco smoking. In stratified analyses, however, a reduced risk was also observed in light drinkers and non-current smokers, making it unlikely that residual confounding explained all of the demonstrated effects of fruits and vegetables. Second, with regard to the validity of the FFQ, the correlation coefficients of 0.57 for fruits and 0.38 for vegetables were not reasonably high. Therefore, some misclassification may have been unavoidable, meaning that a substantial amount of variability for the observed associations might be explained by factors other than fruits and vegetables, such as confounding or chance.

In conclusion, this population-based prospective cohort study in middle-aged Japanese men provides further evidence for the protective role of fruits and vegetables in the development of esophageal SCC. To effectively reduce the burden of this disease, however, priority should be placed on smoking and drinking cessation. Finally, cruciferous vegetables deserve further investigation as foods possibly effective in the prevention of esophageal SCC.

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Appendix

Shown below were the individual items included in each plant food group. Cereals consisted of rice, rice with vitamin supplementation, rice with other grains, rice cake, bread, three types of Japanese noodle (udon, soba and Okinawa soba) and Chinese noodle. Potatoes were composed of potato, sweet potato and yam. Pulses comprised tofu, boiled tofu, yushi-tofu, freeze-dried tofu, deep-fried tofu, fermented soybeans and soy milk. Fungi were three types of Japanese mushroom (shitake, enokitake and shimeji).