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Serum pepsinogens and risk of gastric and esophageal cancers in the General Population Nutrition Intervention Trial cohort

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Abstract

Objective—Low serum pepsinogen I (PGI) and low pepsinogen I/pepsinogen II ratio (PGI/II ratio) are markers of gastric fundic atrophy. We aimed to prospectively test the association between serum PGI/II ratio and risks of gastric noncardia adenocarcinoma, gastric cardia adenocarcinoma, and esophageal squamous cell carcinoma.

Design—Case-cohort study nested in a prospective cohort with over 15 years of follow-up.

Setting—Rural region of the People's Republic of China.

Subjects—Men and women aged 40–69 at study baseline.

Main outcome measures—Adjusted hazard ratios and 95% confidence intervals for the association between serum PGI/II ratio and cancer risk

Results—Compared to subjects with PGI/II ratio of > 4 , those with ≤ 4 had HRs (95% CIs) of 2.72 (1.77–4.20) and 2.12 (1.42–3.16) for noncardia and cardia gastric cancers, respectively. Risk of both cancers were also increased when other cut points ranging from 3 to 6, or when we used quartile models, or nonlinear continuous models. Risk of ESCC was marginally increased in those with PGI/II ratio ≤ 4 , with HR (95% CI) of 1.56 (0.99–2.47), but quartile models and continuous models showed no increased risk. The nonlinear continuous models suggested that any single cut point collapsed subjects with dissimilar gastric cancer risks, and that using cut points was not an efficient use of data in evaluating these associations.

Conclusion—In this prospective study, we found similar and significantly increased risks of noncardia and cardia gastric adenocarcinomas in subjects with low PGI/II ratio, but little evidence for an association with ESCC risk.

Keywords

Gastric cancer; Esophageal cancer; Pepsinogen; Case-cohort

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Competing interests None to declare

Introduction

Pepsinogens are aspartic proteinases which are mainly secreted by gastric cells. They can be immunologically classified into two major types: pepsinogen I (PGI) and pepsinogen II (PGII). [1] PGI is secreted only from the gastric fundic mucosa, while PGII is secreted from the cardiac, fundic, and antral mucosa of the stomach, and also from the duodenal mucosa.[1] Small numbers of these molecules are also released into the circulation and can be measured in serum.

Gastric fundic atrophy is characterized by a change of the fundic mucosa to an antral-type mucosa, [2] and because the antral mucosa does not secrete PGI, people with gastric fundic atrophy have a lower mean serum PGI concentration than those without atrophy. Both mucosal types secrete PGII, however, so serum PGII levels remain stable or are increased during progression from a normal stomach to one with severe atrophy.[3] The net effects of severe atrophy on serum pepsinogen concentrations are lower PGI and a stable or increased PGII, and this leads to a lower PGI/II ratio.[3] Both a low serum PGI concentration and a low serum pepsinogen I/pepsinogen II ratio (PGI/II ratio) have been used as noninvasive markers for gastric fundic atrophy. [3;4] Several recent studies suggest that the PGI/II ratio is a more accurate indicator of atrophy than PGI concentration alone.[5] Most previous studies have used single cut points [6] to define serologic atrophy and the chosen cut points have varied depending on the research group and the population studied.

Prospective, retrospective, and cross-sectional studies have shown that atrophy of the fundic mucosa, diagnosed histologically or by measuring serum pepsinogens, is associated with higher risk of gastric cancer.[7-19] Like the association with atrophy, some studies have suggested that the serum PGI/II ratio may be a better predictor of future gastric cancer risk than serum PGI alone.[9;13;14] Because most previous prospective studies of gastric cancer have had only limited numbers of gastric cardia adenocarcinoma cases,[14-19] currently available data on the association between serum pepsinogens and future gastric cancer risk are based mainly on noncardia cancer cases.

There is also limited data on the association between chronic atrophic gastritis and risk of esophageal squamous cell carcinoma (ESCC). A case-series published in 1993 noted that atrophy was common among subjects with ESCC.[20] A population-based case-control study conducted in Sweden was the first to report that low serum PGI was associated with higher risk of ESCC.[7] These findings were corroborated in a Japanese case-control study which found that gastric atrophy, identified serologically or histologically, was associated with a higher risk of superficial ESCC.[21] One proposed mechanism to explain this association posits that gastric atrophy allows bacterial overgrowth in the stomach and a subsequent increase in *N*-nitroso compound generation.[22] However, a recent prospective study from the Netherlands reported that histologically diagnosed gastric atrophy, intestinal metaplasia, and dysplasia all conferred similar increased risks of ESCC and argued that the lack of progressively higher risk estimates implies a non-causal association.[23] Currently there are no published prospective studies of serum pepsinogens and risk of ESCC.

The people of Linxian, China, have very high rates of ESCC and gastric cardia adenocarcinoma, and also have moderately high rates of gastric noncardia adenocarcinoma; approximately 20% of Linxian residents die of these cancers. This study examines the associations between baseline serum PGI concentration and PGI/II ratio and the incidence of gastric noncardia adenocarcinoma, gastric cardia adenocarcinoma, and ESCC in a long term prospective cohort study conducted in Linxian.

Materials and methods

Study cohort

The subjects of this study were selected from the cohort of all participants in the Linxian General Population Nutrition Intervention Trial. We have previously given a detailed description of the design, conduct, and results of this trial and its extended follow-up.[24;25] In brief, the participants were 29,584 healthy adults aged 40-69 years from four Linxian communes. In the spring of 1985, one year prior to the start of intervention, each participant was interviewed, given a brief physical exam, and had 10 ml of blood drawn. After collection, serum specimens were separated, aliquoted, and stored frozen at -80°C for future analyses. The trial tested the ability of four vitamin/mineral combinations to affect esophageal and gastric cancer incidence and mortality in Linxian. Supplements were taken daily for 5.25 years, from March 1986 through May 1991. Throughout the trial period, local health care workers recorded cancer incidence and mortality data at monthly intervals, and periodic surveys were conducted to verify the completeness and accuracy of this follow-up information. Pathology slides and/or x-rays were available for 85% of the cancer cases in this study, and these were reviewed and the case status confirmed by a panel of American and Chinese experts. For cancer cases without such diagnostic materials and for deaths due to causes other than cancer, reviews were performed by Chinese experts. In the subsequent 10 years after the trial, study subjects were contacted monthly, either by village health workers or by study interviewers and cancer diagnoses were verified by senior Chinese diagnosticians from Beijing. Case ascertainment is considered complete, and loss to follow-up was minimal (< 1%). Outcomes for the present study were based on follow-up data from March 1986 through May 2001.

Informed consent was provided by all subjects and the conduct of the Linxian General Population Nutrition Intervention Trial and extended follow-up was approved by the institutional review boards of the Cancer Institute of the Chinese Academy of Medical Sciences and the US National Cancer Institute.

Cases and subcohort

By May 2001, 1958 ESCC cases, 1089 cases of gastric cardia adenocarcinoma, and 363 cases of gastric noncardia adenocarcinoma were diagnosed. Gastric tumors were defined as cardia cancers if they were centered in the most proximal 3-cm of the stomach and as noncardia cancers if they were centered distal to this region. There were no cases of esophageal adenocarcinoma in the cohort during the follow-up period.

We used a case-cohort design[26] to select subjects for the PGI and PGII assays. We chose this design over a nested case-control design because this study had three outcomes and in a case-cohort analysis the subcohort could be used as a comparison group for all three. In addition, using this design, the prevalence of the exposures (PGI and PGI/II ratio) in the entire cohort could be estimated from the subcohort.

We selected a random sample of 300 ESCC cases and 600 cardia cancer cases and all 363 noncardia cancer cases as case subjects. For the subcohort comparison group, we selected a random sample of 1050 subjects from the entire baseline cohort. Serum samples were available for 2066 (89%) of the selected subjects, including 261 selected ESCC cases, 517 selected cardia cancer cases, 314 selected noncardia cancer cases, and 974 subcohort members. Because the subcohort was a random sample of the cohort, some subcohort members developed cancer during the follow-up period. Therefore, the total number of cancer cases was equal to the number of cancer subjects selected as cases plus the number of cancer cases that developed within the subcohort. The final numbers of cases in this study were 323 ESCC cases (261

selected cases and 62 subcohort cases), 546 cardia cancer cases (517 selected cases and 29 subcohort cases), and 330 noncardia cancer cases (314 selected cases and 16 subcohort cases).

Serologic assays

Serum PGI and PGII were measured by enzyme-linked immunosorbent assays (Biohit ELISA kit, Finland) which were performed by experienced technicians who were unaware of the subjects' case-control status. Serum was measured in duplicate and then the average value was used for each individual. When there were large differences in the duplicate results (a total of 5 subjects), an additional assay was conducted and the average of the two closer measurements was considered the final result. Duplicate results were strongly correlated; Pearson's correlation coefficient was 0.995 for PGI and 0.997 for PGII.

Two duplicate quality control samples that were provided with the kits were included on each assayed plate. Using all these QC samples together, the coefficients of variation were 6.5% and 2.7% for the PGI and PGII assays, respectively. In addition, 103 quality control samples, aliquoted from a single large serum pool from the National Cancer Hospital, CICAMS, Beijing, were distributed among the 54 assay plates. On the basis of all these QC samples together, the coefficients of variation were 5.5% and 6.7% for PGI and PGII, respectively. We previously published a description of the generation and analysis of the data on *Helicobacter pylori* serology in these subjects.[27]

Statistical methods

Correlation coefficients were calculated using Pearson parametric methods and means were compared using t-tests. For the primary risk analyses we used Cox proportional hazards models to estimate crude and adjusted hazard ratios (HRs) and 95% confidence intervals (95% CIs). Models were fit using the case-cohort Cox models available in Epicure Software (Hirosoft, Seattle, WA). Serum PGI and PGI/II ratio were the main exposures of interest and they were analyzed as dichotomous variables using single cut points. Seropositivity cut points were defined as ≤ 50 $\mu\text{g/L}$ for PGI and $\leq 3, 4, 5$, or 6 for PGI/II ratio. In addition to these dichotomous analyses, we evaluated the association of pepsinogen values and cancer outcomes by quartile analyses, based on the distributions of the serum markers in the subcohort, and by nonlinear continuous models of the PGI/II ratio. The nonlinear continuous models were fit using PROC GAM in SAS v9 (Cary, NC) and odds ratios were calculated relative to the 87.5th percentile (the median of the fourth quartile) of the distribution of the PGI/II ratio in the subcohort. For the GAM models we used a loess smoother and the generalized cross validation method to select the degrees of freedom. All reported *P*-values are two-sided. We considered *P*-values < 0.05 or confidence intervals that exclude 1.0 statistically significant. The proportional hazards assumption was examined graphically and tested using time interaction terms.

Consistent with previous studies of upper gastrointestinal cancers in Linxian, age (years), sex (male vs. female), history of smoking (yes vs. no), alcohol consumption (yes vs. no in the past 12 months), and body mass index (Kg/m^2) were considered as potential confounders. In addition, *Helicobacter pylori* seropositivity (defined as either whole cell or CagA seropositivity) was added to the list of potential confounders. Medians and interquartile ranges of continuous variables (age and body mass index), and numbers and percentages of categorical variables (sex, history of smoking, history of alcohol consumption, and *H. pylori* seropositivity) were calculated and reported for the subcohort and each cancer type to assess the differences by case status.

We tested for interactions by age, sex, and assignment to each of the four trial treatment factors. There were no significant interactions. Because previous studies have suggested an interaction

between atrophy and *H. pylori* seropositivity in risk of gastric cardia cancer [28] and noncardia cancer [17], we also tested for an interaction by *H. pylori* status and report the results.

Results

Table 1 shows the demographic characteristics, potential confounders, and serum pepsinogen concentrations in cancer cases and subcohort members. Compared to the subcohort, all subgroups of cancer cases were older. Gastric noncardia and cardia cancer cases were more likely to be men, to smoke, and to be *H. pylori* seropositive than the subcohort members. The prevalence of alcohol drinking and the mean body mass index were similar among all subgroups. The frequencies of these variables by case status were similar to those previously reported for the full cohort.[29]

The Pearson correlation coefficient for the association between PGI and PGII among the subcohort members was 0.67. We also found that both PGI and PGII serum concentrations differed by *H. pylori* seropositivity in the subcohort. The mean (SD) PGI and PGII concentrations ($\mu\text{g/L}$) and PGI/II ratio were 99.6 (35.9), 7.9 (6.4), and 16.7 (11.1) in those without *H. pylori* seropositivity compared to 130.9 (54.3), 19.6 (12.2), and 9.3 (8.6) in those with *H. pylori* seropositivity, respectively and all three *P*-values were <0.0001 .

Table 2 shows the number of subjects with serologic atrophy, defined by the PGI and PGI/II ratio cut points, for the subcohort and each cancer site. The prevalence of serologic atrophy in the subcohort was 3.9% when defined as $\text{PGI} \leq 50 \mu\text{g/L}$. PGI/II ratios of ≤ 3 , 4, 5, or 6 resulted in prevalences of 5.8%, 7.5%, 15%, and 26%, respectively.

For gastric noncardia adenocarcinoma, the adjusted HR (95% CI) for having serum $\text{PGI} \leq 50 \mu\text{g/L}$ was 1.87 (0.98-3.56). Comparing those with $\text{PGI/II ratio} \leq 4$ to those > 4 for serum PGI/II ratio, we found that a low PGI/II ratio had an adjusted HR (95% CI) of 2.72 (1.77-4.20). When we used other cut points (3, 5, and 6), we found similarly increased risks (adjusted HRs 2.17, 2.67, and 1.94, respectively). Risk of gastric cardia adenocarcinoma was also increased in those with low serum PGI/II ratio, with magnitudes and patterns similar to noncardia cancer. The adjusted HR (95% CI) for a $\text{PGI/II} \leq 4$ was 2.12 (1.42-3.16) but risk was increased when we used other cutpoints too. For ESCC, using a cut point of 4 for PGI/II ratio, the risk was marginally non-significantly increased (adjusted HR (95% CI) of 1.56 (0.99-2.47)), but HRs were close to 1 for other cut points.

Because there was no obviously superior cut point, we evaluated the association between serum pepsinogens and risk of gastric and esophageal cancers by fitting models using quartiles defined by the distributions of PGI or the PGI/II ratio in the subcohort (Table 3). When we used these models, we found that the associations between serum pepsinogens and risk of both subsites of gastric cancer were present across the full distribution of pepsinogen values, and did not suggest a threshold effect, as is implied when using dichotomous models. The estimates across PGI/II quartiles for noncardia gastric adenocarcinoma had a significant test for trend ($P=2.7 \times 10^{-5}$) and risks of 0.88, 1.33, and 2.16 in quartiles 3, 2, and 1 compared to quartile 4. A similar pattern was present for gastric cardia adenocarcinoma with a significant trend test ($P=1.1 \times 10^{-4}$) and increasing risks. Compared to quartile 4 those in quartile 1 had a HR (95% CI) of 1.73 (1.20-2.51). In contrast, there was no apparent association between serum pepsinogens and ESCC when modeled using pepsinogen quartiles with a non-significant *P* for trend (0.53) and all HR estimates below 1.0.

Next, we used nonlinear continuous models to graphically explore the association between the serum PGI/II ratio and risk of gastric and esophageal cancers. We calculated and plotted odds ratios for different values of the serum PGI/II ratio compared to a PGI/II ratio of 17.8, the 87.5th percentile (the median of the fourth quartile) of the subcohort. Figure 1 shows the

association between PGI/II ratio and the risk of gastric and esophageal cancers. We found a strong and progressively increasing risk of gastric noncardia cancer with decreasing serum PGI/II. We found similar results for gastric cardia adenocarcinoma. In contrast to the gastric cancers and consistent with the quartile models, we found no evidence of an association between PGI/II ratio and risk of ESCC using these nonlinear continuous models.

Results of analyses by time from serum collection to diagnosis are presented in Table 4. There were statistically significant differences in HRs for the association between low serum PGI and gastric noncardia cancer diagnosed in the three time periods. The adjusted HR (95% CI) decreased from 3.49 (1.69 – 7.22) in the trial period (≤ 5.25 years), to 1.55 (0.47 – 5.17) in the first post-trial follow-up period (5.26 – 10 years), and then to 0.31 (0.03 – 2.84) in the second post-trial follow-up period (> 10 years), and the P-value for difference was 0.03. No statistically significant differences were observed for HRs of other associations across the three time periods, with all P-values ≥ 0.27 .

We examined the combined effects of atrophy (defined as $\text{PGI/II} \leq 4$) and baseline *H. pylori* seropositivity on risk of each cancer (Table 5). For gastric noncardia adenocarcinoma, atrophy increased risk more in those who were *H. pylori* seropositive rather than in those who were *H. pylori* seronegative but the interaction term was not statistically significant (P for interaction = 0.13). For gastric cardia adenocarcinoma, atrophy approximately doubled risk in both those with and without *H. pylori* (P for interaction = 0.87). For ESCC, the HRs for atrophy were similar in those with and without *H. pylori* (P for interaction = 0.77).

Discussion

In this case-cohort study, we prospectively evaluated the association between gastric fundic atrophy, as estimated by baseline serum pepsinogen levels, and future development of gastric and esophageal cancers in the General Population Nutrition Intervention Trial cohort in Linxian, China. We found an association between low serum PGI or PGI/II ratio and subsequent risk of gastric noncardia and cardia adenocarcinomas, but little evidence of an association between these markers and increased risk of ESCC. This is the largest evaluation to date of the association of gastric atrophy and gastric cardia adenocarcinoma, and it is the first prospective evaluation of the association of gastric atrophy and esophageal squamous cell carcinoma.

Most previous studies of the association between serum pepsinogens and gastric or esophageal cancer risk have used dichotomous comparisons based on single cut points, generally $\text{PGI} \leq 70$ -30, $\text{PGI/II} < 3$, or a combination of these cut points.[6] We tested different PGI/II ratio cut points because of the relatively high median pepsinogen levels in our population. Higher serum pepsinogen concentrations have also been reported in other Asian populations.[6] But in our quartile analyses (Table 3) and nonlinear continuous analyses (Figure 1) we found no evidence of thresholds or logical cut points. Indeed, our results suggest that dichotomous comparisons are not an efficient use of data in estimating associations between serum pepsinogen concentrations and risk of gastric cancer and that these comparisons may be misleading because any cut point will collapse people at considerably dissimilar risk into the same category.

Another interesting finding in this study was the strong correlation found between PGI and PGII (Pearson's $r = 0.67$). Previous studies have also found similar strong correlation coefficients between PGI and PGII [3;9;30], perhaps because both peptides are secreted by the fundic cells of the stomach. These findings are consistent with the hypothesis that the PGI/II ratio takes into account both the fact of fundic atrophy (via the PGI component) and the total secretion capacity of pepsinogens in an individual (via the PGII component).

Previous studies have consistently shown that low serum PGI or a low serum PGI/II ratio are associated with higher risk of gastric noncardia cancer.[31] Our findings concur with these previous results. Also similar to most previous studies, we found that the PGI/II ratio was a stronger predictor of risk than PGI alone, even in models adjusted for *H. pylori* seropositivity.

There are few previous studies on the association of serum PGI or the PGI/II ratio with gastric cardia adenocarcinoma. Most previous prospective studies have had very limited numbers of gastric cardia cancer cases, so they have not reported pepsinogen data separately for cardia cancers.[16;17;19] Some prospective studies, however, with moderate numbers of cardia cancer cases (ranging between 23 and 44), have reported on these associations, and all of these studies have shown increased cancer risk among subjects with low serum PGI or PGI/II ratios, with relative risks (95% CI) of 5.0 (0.6 – 42.8) [15], 4.11 (1.42 – 11.9) [18], and 1.60 (0.62 – 4.14).[28] Likewise, three case-control studies that examined these same associations all found increased risk of gastric cardia cancer associated with low serum PGI or PGI/II values.[7;9; 11] Of note, all studies reporting the magnitude of the association for both sites have found similar odds ratios for cardia and noncardia cancers. Our results confirm these previous findings, and show that gastric atrophy increases risk of both cardia and noncardia gastric cancers in Linxian, with similar magnitudes and association patterns at these two cancer sites.

Two recent studies, one from Norway [28] and one from Iran [32], showed that the risk of cardia cancer associated with atrophy may be modified by *H. pylori* status. However, our results did not show any statistically significant interaction between atrophy and *H. pylori* in causing cardia cancer. Also, another study from Japan suggested that risk of gastric cancer was highest in those who had atrophy but were seronegative for *H. pylori* antibodies.[17] In contrast, in our study, the highest risks were observed among those who had atrophy and were seropositive for *H. pylori* antibodies. These differences in results may be related to chance findings because of small sample sizes or may be due to differences between study populations. The numbers of cardia cancer cases in the Norwegian and Iranian studies were 44 and 53, respectively. Also, the total number of gastric cancer cases in the Japanese study was 43. In our study area the cardia cancer rates are unusually high and are a large proportion of gastric cancer cases (approximately 75% of all gastric cancers); cardia cancer co-occurs with the very high rates of ESCC; and the risk of cardia cancer is increased among those who are seropositive for *H. pylori*. [27] Therefore, population-specific findings for cardia cancer are also possible.

Ye and colleagues, in a case-control study published in 2004, were the first to report an association between low serum pepsinogen levels and ESCC risk [7], and others have since reported that an association is present in Japanese [21] and Dutch populations.[23] When we analyzed our data dichotomously, we also found a borderline insignificantly increased risk of ESCC associated with low serum PGI/II ratio. But, unlike the associations seen with gastric cardia and noncardia cancer, there was no evidence of an increasing trend in HRs across the exposure quartiles and no evidence of an association in nonlinear continuous models. When we stratified on follow-up time it showed an apparently stronger association with ESCC in the first 5.25 years of follow-up (the trial intervention period) than in the subsequent 10 years of follow-up. de Vries *et al.* reported a similar phenomenon in their cohort.[23] The Dutch study investigators attribute their result to selection bias, but this can't be the explanation in our study design. Overall, our prospective study finds little evidence of an association between serologic markers of gastric atrophy and ESCC. But, we note that in a separate analysis using a different group of subjects in Linxian, we found a strong linear association between serum PGI/II ratio and risk of esophageal squamous dysplasia, the precursor lesion for ESCC.[30]

We believe that our findings suggest that pepsinogen concentrations alone will not be useful for cancer screening. Low serum PGI and PGI/II ratio levels predict higher risks of gastric cardia and noncardia cancers, and perhaps higher risk of ESCC as well, but as Stemmermann

and colleagues have commented,[14] these measurements are not sensitive enough to be used for screening. Low serum PGI and a low PGI/II ratio have been reported to be associated with 2-10 fold increased risk of gastric cancer, but successful screening techniques are usually associated with much larger relative risks.[33] For example, a marker with a sensitivity and specificity of 95% has an odds ratio of 361. This is also reflected in the newest Japanese Guidelines for Gastric Cancer Screening that do not recommend serologic testing for serum pepsinogens.[34]

The strengths of this study include its prospective design, a large sample size, essentially complete follow-up, modeling the associations using cut points, quartiles, and nonlinear continuous models, and the availability of data on potential confounders. Furthermore, our study was conducted in a population at high risk for the diseases in question, where the results are most relevant. The results may or may not be generalizable to Western populations, which have a lower incidence of these cancers and where cardia cancer may have a different etiology from those studied in Asian populations.[35]

In summary, this prospective study confirmed a strong association between gastric fundic atrophy, as estimated by low serum PGI and PGI/II ratio, and subsequent risk of cardia and noncardia gastric cancers. There was little evidence of an association with an increased risk of ESCC.

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Figure 1. Nonlinear continuous models of the association between serum PGI/II ratio and risk of upper gastrointestinal cancers in the Linxian General Population Nutrition Intervention Trial cohort

The association (odds ratio) was calculated compared to the median of the fourth quartile of the PGI/II ratio in the subcohort (a PGI/II ratio of 17.8) using a generalized additive model with loess smoother adjusted for age, sex, cigarette smoking, alcohol consumption, body mass index, and *H. pylori* seropositivity.

Demographic characteristics, tobacco use, alcohol consumption, *H. pylori* seropositivity and serum pepsinogen concentrations in cases and the subcohort of the Linxian General Population Nutrition Intervention Trial cohort

Table 1

	Subcohort(n=974)	Gastric noncardia adenocarcinoma(n=330)	Gastric cardia adenocarcinoma(n=546)	Esophageal squamous cell carcinoma (n=323)
Age in years, median (IQR)	52 (44-59)	57 (50-62)	56 (30-62)	56 (48-61)
Males, N (%)	443 (45.5)	216 (65.5)	326 (59.7)	147 (45.5)
Tobacco smokers, N (%) ¹	326 (33.5)	154 (46.7)	224 (41.0)	105 (32.5)
Alcohol drinker, N (%) ²	236 (24.2)	78 (23.6)	136 (24.9)	63 (19.5)
BMI (kg/m ²), median (IQR)	22 (20-23)	21 (20-23)	22 (20-23)	21 (20-23)
<i>H. pylori</i> seropositives, N (%)	713 (73.2)	265 (80.3)	445 (81.5)	245 (75.9)
Pepsinogen I, µg/L, median (IQR)	118 (87-152)	119 (85-149)	121 (96-152)	118 (85-150)
Pepsinogen II, µg/L, median (IQR)	17 (16-23)	18 (10-27)	18 (10-26)	15 (7.0-24)
Pepsinogen I:II ratio, median (IQR)	8.6 (5.9-13)	6.6 (4.6-11)	6.8 (5.0-11.0)	8.8 (5.7-14)

¹ Ever smoking cigarettes for six or more months

² Ever drinking any alcoholic beverage in the last 12 months

Table 2

Hazard ratios and 95% confidence intervals for the association between dichotomized serum pepsinogen levels and risk of upper gastrointestinal cancers in the Linxian General Population Nutrition Intervention Trial cohort

Cancer type and exposure	Serologic atrophy, N (%)	Crude model ¹ HR (95% CI)	Adjusted model ^{1,2} HR (95% CI)
Subcohort			
PGI (≤ 50 $\mu\text{g/L}$)	38 (3.9%)		NA
PG I/II ratio (≤ 3)	44 (4.5%)		
PG I/II ratio (≤ 4)	73 (7.5%)		
PG I/II ratio (≤ 5)	141 (15%)		
PG I/II ratio (≤ 6)	250 (26%)		
Gastric noncardia adenocarcinoma			
PGI (≤ 50 $\mu\text{g/L}$)	19 (5.8%)	1.52 (0.86-2.69)	1.87 (0.98-3.56)
PG I/II ratio (≤ 3)	34 (10%)	2.71 (1.68-4.37)	2.17 (1.26-3.74)
PG I/II ratio (≤ 4)	61 (19%)	3.29 (2.25-4.81)	2.72 (1.77-4.20)
PG I/II ratio (≤ 5)	99 (30%)	2.67 (1.98-3.61)	2.67 (1.89-3.78)
PG I/II ratio (≤ 6)	139 (42%)	2.16 (1.65-2.81)	1.94 (1.42-2.65)
Gastric cardia adenocarcinoma			
PGI (≤ 50 $\mu\text{g/L}$)	26 (4.8%)	1.24 (0.74-2.08)	1.74 (0.99-3.06)
PG I/II ratio (≤ 3)	37 (6.8%)	1.70 (1.07-2.71)	1.58 (0.95-2.63)
PG I/II ratio (≤ 4)	71 (13%)	2.16 (1.51-3.10)	2.12 (1.42-3.16)
PG I/II ratio (≤ 5)	139 (25%)	2.12 (1.62-2.78)	2.19 (1.61-2.97)
PG I/II ratio (≤ 6)	212 (39%)	1.87 (1.49-2.35)	1.65 (1.27-2.16)
Esophageal squamous cell carcinoma			
PGI (≤ 50 $\mu\text{g/L}$)	17 (5.3%)	1.38 (0.78-2.46)	1.64 (0.89-3.00)
PG I/II ratio (≤ 3)	15 (4.6%)	1.15 (0.63-2.08)	1.10 (0.59-2.04)
PG I/II ratio (≤ 4)	32 (9.9%)	1.60 (1.03-2.47)	1.56 (0.99-2.47)
PG I/II ratio (≤ 5)	57 (18%)	1.34 (0.96-1.87)	1.37 (0.96-1.95)
PG I/II ratio (≤ 6)	91 (28%)	1.16 (0.88-1.54)	1.07 (0.79-1.45)

¹ Hazard ratios (HRs) and 95% confidence intervals (95% CIs) were obtained from Cox regression models.

² Models were adjusted for age, sex, cigarette smoking (ever versus never), alcohol consumption (any versus none), body mass index, and *H. pylori* seropositivity.

Table 3

Hazard ratios and 95% confidence intervals for the association between serum pepsinogen quartiles and risk of upper gastrointestinal cancers in the Linxian General Population Nutrition Intervention Trial cohort¹

Cancer type and exposure	Quartile 4	Quartile 3 HR (95% CI)	Quartile 2 HR (95% CI)	Quartile 1 HR (95% CI)	P-value for trend test ²
Gastric noncardia adenocarcinoma					
PGI	1.00	1.27 (0.87-1.86)	1.46 (0.96-2.21)	2.38 (1.53-3.71)	8.0×10^{-5}
PG I/II ratio	1.00	0.88 (0.57-1.38)	1.33 (0.84-2.13)	2.16 (1.38-3.38)	2.7×10^{-5}
Gastric cardia adenocarcinoma					
PGI	1.00	1.19 (0.86-1.63)	1.51 (1.08-2.13)	1.33 (0.91-1.96)	0.041
PG I/II ratio	1.00	0.79 (0.55-1.15)	1.30 (0.89-1.91)	1.73 (1.20-2.51)	1.1×10^{-4}
Esophageal squamous cell carcinoma					
PGI	1.00	1.06 (0.74-1.51)	0.97 (0.66-1.43)	1.37 (0.92-2.06)	0.20
PG I/II ratio	1.00	0.75 (0.52-1.09)	0.72 (0.48-1.08)	0.86 (0.58-1.27)	0.53

¹ Hazard ratios (HRs) and 95% confidence intervals (95% CIs) were obtained from Cox regression models adjusted for age, sex, cigarette smoking, alcohol consumption, body mass index, and *H. pylori* seropositivity. Quartile 4 was the referent category for all analyses and the quartile boundaries were defined as the 75th, 50th, and 25th percentiles in the subcohort. For PGI these cut points were 152, 118, and 87 and for PGI/II these were, 13, 8.6, and 5.9, respectively.

² P-values for trend were obtained from score tests in adjusted models with an added variable that assigned the ordinal quartile values (1-4).

Table 4

Hazard ratios and 95% confidence intervals for the association between serum pepsinogen levels and risk of upper gastrointestinal cancers in the Linxian General Population Nutrition Intervention Trial cohort, by follow-up time¹

Cancer type and exposure	Time from serum collection to cancer diagnosis			P-value for difference ²
	≤5.25 years HR (95% CI)	>5.25 – 10 years HR (95% CI)	>10 years HR (95% CI)	
Gastric noncardia adenocarcinoma PGI	3.49 (1.69-7.22)	1.55 (0.47-5.17)	0.31 (0.03-2.84)	0.03
PG I/II ratio(≤4)	2.40 (1.00-5.76)	2.56 (1.14-5.72)	2.08 (0.78-5.57)	0.62
Gastric cardia adenocarcinoma PGI	1.50 (0.71-3.20)	2.48 (0.88-6.99)	1.29 (0.34-4.81)	0.64
PG I/II ratio(≤4)	2.19 (1.35-3.54)	2.04 (0.93-4.46)	2.09 (0.82-5.34)	0.99
Esophageal squamous cell carcinoma PGI	2.46 (1.18-5.15)	0.61 (0.13-2.89)	1.64 (0.49-5.53)	0.27
PG I/II ratio(≤4)	1.51 (0.81-2.84)	1.49 (0.63-3.55)	1.71 (0.67-4.33)	0.97

¹ Hazard ratios (HRs) and 95% confidence intervals (95% CIs) were obtained from Cox regression models adjusted for age, sex, cigarette smoking (ever versus never), alcohol consumption (any versus none), body mass index, and *H. pylori* seropositivity.

² P-values for difference were obtained from 2 degree-of-freedom score tests that compared adjusted models with a single term for exposure to models with three terms, one for each follow-up period.

Table 5

Hazard ratios and 95% confidence intervals for the association between serum pepsinogen levels and risk of upper gastrointestinal cancers in the Linxian General Population Nutrition Intervention Trial cohort, by baseline *H. pylori* status¹

Cancer type and exposure	<i>H. pylori</i> Negative		<i>H. pylori</i> Positive	<i>P</i> -value for interaction ²
Gastric noncardia adenocarcinoma <i>PG I/II ratio</i> (>4)	1.00		1.32 (0.93-1.86)	0.13
<i>PG I/II ratio</i> (≤4)	0.99 (0.22-4.44)		3.98 (2.37-6.66)	
Gastric cardia adenocarcinoma <i>PG I/II ratio</i> (>4)	1.00		1.59 (1.18-2.13)	0.87
<i>PG I/II ratio</i> (≤4)	2.30 (0.79-6.70)		3.32 (2.05-5.38)	
Esophageal squamous cell carcinoma <i>PG I/II ratio</i> (>4)	1.00		1.09 (0.80-1.49)	0.77
<i>PG I/II ratio</i> (≤4)	1.83 (0.57-5.85)		1.66 (0.97-2.84)	

¹ Hazard ratios (HRs) and 95% confidence intervals (95% CIs) were obtained from Cox regression models adjusted for age, sex, cigarette smoking (ever versus never), alcohol consumption (any versus none), body mass index.

² *P*-values for interaction were obtained from Wald tests.