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Prospective Study of Serum Cysteine Levels and Oesophageal and Gastric Cancers in China

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Abstract

Background—Cancers of the upper gastrointestinal tract remain a significant cause of morbidity and mortality. Cysteine, known to be involved in a myriad of immuno-modulatory, anti-oxidant, and anti-carcinogenic pathways, has not been investigated in the aetiology of oesophageal or gastric cancers. To examine the relationship between serum cysteine concentration and risk of these cancers we conducted a nested case-cohort study within the General Population Nutrition Intervention Trial in Linxian, China.

Methods—498 oesophageal squamous cell carcinomas (OSCC) and 255 gastric cardia adenocarcinomas (GCA) were matched by age and sex to 947 individuals from the wider cohort. We calculated hazard ratios (HR) and 95% confidence intervals (95% CI) using the case-cohort estimator for the Cox proportional hazards models, stratified on age and sex, with adjustment for potential confounders.

Results—Higher concentrations of serum cysteine were significantly associated with a lower risk of both OSCC and GCA. For those in the highest quartile of serum cysteine, compared to those in the lowest, the multivariate HRs were 0.70 for OSCC (95% CI: 0.51, 0.98) and 0.59 for GCA (95% CI: 0.38, 0.91). These associations were dose dependent (*P* for trend = 0.006 and 0.008, respectively). These inverse associations were not significantly modified by other risk factors, with the exception of age, where a stronger association was noted among persons in the older age strata.

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Conclusion—Higher serum concentrations of cysteine were associated with a significantly reduced risk of OSCC and GCA. Cysteine should be further investigated for its potential as a chemopreventive agent for upper gastrointestinal cancers.

Keywords

oesophageal squamous cell carcinoma; gastric cardia cancer; hazard ratio; cysteine

Introduction

Cysteine, a non-essential amino acid and metabolite of methionine, is an essential component of the one-carbon metabolism pathway that has been extensively investigated for its role in the synthesis and methylation of DNA and its possible role in carcinogenesis [1, 2]. Cysteine can be synthesized by the body under normal physiological conditions, if a sufficient quantity of methionine is available; most high-protein foods, such as pork, chicken, turkey, eggs, milk and yogurt, contain cysteine. Cysteine has many functions besides its role in one-carbon metabolism. A multi-faceted amino-acid, cysteine can modulate the immune response by inhibiting activation of nuclear factor- κ B (NF- κ B) and the expression of several NF- κ B dependent genes [3]. It can also stimulate proliferation of lymphocytes and T-cell clones under experimental conditions [3]. Evidence from both human and animal studies suggests that cysteine also exerts direct anti-carcinogenic properties, since it can bind to and detoxify acetaldehyde, a known mutagen and carcinogen [4, 5]. A recent study involving rats exposed to environmental cigarette smoke showed that orally administered *N*-acetylcysteine effectively modulated smoke-induced alterations of microRNA expression in rat lungs [6]; specifically, *N*-acetylcysteine modulated microRNAs involved in NF- κ B-mediated stress and P53 functions. Further, cysteine availability is a rate-limiting step in the formation of glutathione (γ -glutamyl-cysteinyl-glycine), an antioxidant tri-peptide with critical roles in nutrient metabolism and wider homeostasis (gene expression, DNA and protein synthesis, cell proliferation and apoptosis, signal transduction, cytokine production and immune response and protein glutathionylation) [7, 8]. Glutathione may also modulate cell death in that high intracellular glutathione levels prevent cell death where low levels enhance it, so that glutathione depletion can activate apoptosis [9]. Immune activation may also be modulated by glutathione: it is essential for activation of T-lymphocytes, polymorphonuclear leukocytes and cytokine production [10].

Cancers of the upper gastrointestinal tract remain a significant cause of morbidity and mortality. In 2002 there were an estimated 934,000 new cases of stomach cancer and 462,000 cases of oesophageal cancer, globally, and with a poor prognosis for both cancers, they ranked as the second and sixth leading causes of cancer-related mortality worldwide [11]. In Linxian, China, where this study was based, cancer of the upper gastrointestinal tract is endemic; mortality rates for oesophageal cancer in Linxian exceed the Chinese average by 10-fold and the American average (for white men) by 100-fold [12].

Cysteine is involved in a range of pathways relevant to carcinogenesis, yet there is a striking dearth of epidemiological studies investigating its role in the aetiology of cancer. To our knowledge, plasma cysteine concentrations have previously been measured only in the context of breast and cervical cancers [13, 14, 15]; given the potential relevance of cysteine to inflammatory and other pathways involved in both oesophageal and gastric carcinogenesis, we investigated the association between serum cysteine concentration and risk of oesophageal squamous cell carcinoma and gastric cardia adenocarcinoma in a case-cohort study nested in the General Population Nutrition Intervention Trial in Linxian, China.

Methods

Subjects in this study were selected from the participants of the Linxian General Population Nutrition Intervention Trial. Details of the design, intervention agents, conduct, and primary end-point analyses of this trial have been published elsewhere [16, 17]. Briefly, the 29,584 healthy adult trial participants were aged between 40–69 years and came from four Linxian communes. One year prior to the intervention each participant was interviewed, given a brief physical examination and had 10mL of blood drawn. The intervention began in March 1986 and continued through May 1991. Participants were randomly assigned to receive either a vitamin-mineral combination or a placebo, in accordance with the partial factorial design of the trial. Four different vitamin-mineral combinations were tested: Factor A (10,000 IU of vitamin A and 45mg of zinc oxide), Factor B (52mg of riboflavin and 40mg of niacin), Factor C (180mg of ascorbic acid and 30µg of molybdenum), and Factor D (50µg of yeast selenium, 15mg of β-carotene, and 30mg of α-tocopherol). Mortality and possible symptoms of cancer among trial participants were ascertained by local village doctors through monthly follow-up, and < 1% of the participants were lost to follow-up during the study period. Diagnoses of cancer were made at local commune and county hospitals and supplemented by a study team which provided clinical and diagnostic services, including endoscopy, for patients with symptoms suggestive of oesophageal or gastric cancer. A panel of U.S. and Chinese experts reviewed the diagnostic material for 85% of the cancer cases in this study. Cancers in the most proximal 3cm of the stomach were defined as cardia cancers and those originating elsewhere in the stomach were designated non-cardia cancers. Ninety-five percent of the anatomic localizations were made with the use of endoscopy, surgery, and/or x-rays. Senior Chinese diagnosticians conducted reviews for cancer cases without diagnostic materials and for deaths due to causes other than cancer. Written informed consent was obtained from each participant prior to enrolment, and human subject protection procedures approved by the institutional review boards of the U.S. National Institutes of Health and the Chinese Academy of Medical Sciences were followed throughout the trial.

Selection of Study Participants for Serum Cysteine Measurement

We used a stratified case-cohort design [18, 19, 20], to select individuals for serum measurements from the cohort of all participants in the General Population Trial. During the 5.25 years of the trial, 640 (oesophageal squamous cell carcinomas) OSCC and 435 gastric cardia adenocarcinomas (GCA) were identified [17]. Following a series of simulations and power calculations, 498 OSCC and 255 GCA were randomly selected from the identified cases for inclusion in this analysis (see Table 1). Serum cysteine was also measured in a stratified random sample of all trial participants (n=947, also selected following power calculations), without regard to outcome, as the comparison group (referred to as the sub-cohort). Six strata were defined by sex and the following age categories (at the start of intervention): <50 years old; 50–60 years old; and >60 years old. Cohort subjects were drawn from each stratum to achieve a greater than 1:1 ratio for control subjects to case subjects for both OSCC and GCA combined. The lowest within-stratum ratios of control subjects to case subjects for incident OSCC or GCA were 1.4 and 2.7, respectively. Overall, we measured serum cysteine concentrations in 947 subcohort subjects and 753 case subjects.

Laboratory Analysis

At the time of baseline interview (March-May 1985: one year before the initiation of the intervention), a 10ml venous blood sample was collected from all consenting participants. Samples were stored 3–6 hours on ice during transportation to the central field station where they were separated, aliquotted and stored at –45°C for 3–4 days before being shipped to Beijing for long-term storage at –85°C. In 1996, aliquots of these baseline sera were transferred to the National Cancer Institute repository on dry ice. Samples selected for

inclusion in the cysteine analysis were thawed, and smaller aliquots were made, immediately refrozen and shipped on dry ice to the analytic laboratories (Jean Mayer U.S. Department of Agriculture Human Nutrition Research Center on Aging at Tufts University, Boston, MA).

Serum cysteine concentration was determined using a modified high performance liquid chromatography (HPLC) method [21]. Each batch contained adjacent blinded case and matched control samples, as well as six blinded quality control samples derived from a pool of serum from Linxian residents. The overall coefficient of variation in the 153 QC samples was 13%. Serum creatinine was also measured, and its overall coefficient of variation in the QC samples was 12%.

Statistical Analysis

Mean and quantile values for serum cysteine were calculated using the sampling weights for the whole General Population Trial cohort, so that estimates are derived from the entire cohort, including those who may have later developed cancer. We used multivariable linear regression to examine the relationships between serum cysteine concentration and its possible predictors. In this linear regression model age was centred at 55 years (the middle of the sub-cohort age range) and body mass index (BMI, [weight in kg]/[height in m]²) was centred at the sub-cohort median, 22 Kg/m².

Follow-up time was measured from the start of the intervention, in March 1986, to any cancer diagnosis, loss to follow-up, or death. Hazard ratios (HRs) and 95% confidence intervals (CIs) were calculated using the case-cohort estimator for the Cox proportional hazards models [18, 19, 20, 22]. All estimates come from models stratified on the six sex-age sampling strata. Adjustment for variation within age strata was made using stratum-specific age terms for continuous age. The proportionality assumption was examined using models that allowed time-dependent HRs and no evidence was found that HRs varied with time.

The relationship between cysteine and risk of OSCC or GCA was examined using a number of alternative metrics. Continuous estimates of cysteine were scaled to 19 nmol/mL (1/2 the interquartile range: (75th percentile-25th percentile) ÷ 2). HRs were also estimated according to quartiles and ordinal values were assigned to the quartiles for trend tests.

Multivariate models were stratified on the 6 sex and age group categories used in the subcohort selection. Additional adjustments included separate continuous age variables for each stratum and variables for ever smoking, any alcohol intake in the previous 12 months, BMI (measured height and weight), serum creatinine (mg/dL) and serum creatinine squared (because of a non-linear association between creatinine and cysteine). Stratified models were also examined for sex, age, cigarette smoking and alcohol drinking. These stratified models included an interaction term (cross-product) and were fully adjusted for all other terms. As cigarette and alcohol consumption were almost completely restricted to men in this cohort, these stratified models are not presented for women.

To investigate the possibility the pre-clinical cancers may affect serum cysteine concentrations we examined whether the HR associated with cancer that developed in the first 2 years of the study differed from the HR associated with cancer that developed after 2 years. These differences were not significant for either OSCC ($P=0.70$) or GCA ($P=0.89$). The randomized nature of the original intervention meant that confounding by intervention status was unlikely; possible interaction between serum cysteine concentration and the intervention was examined and this was not significant.

Results

Baseline characteristics of the cases and sub-cohort are shown in Table 1. Incident cancer cases included 498 OSCC and 255 GCA. There were almost twice as many GCA cases in men than in women, but the number of OSCC cases was about equal in men and women. The associations between smoking, alcohol and BMI and both gastric and oesophageal cancers have been previously reported for this population [23].

The median (and 25th and 75th percentile) serum cysteine concentrations (in nmol/mL) in the cases and cohort participants are shown in Table 2. The median serum cysteine concentration for the cohort was 196 nmol/mL (IQR: 178–216 nmol/mL), with no significant difference between males (median: 196 nmol/mL; IQR: 179–215 nmol/mL) and females (median: 196 nmol/mL; IQR: 176–217 nmol/mL). With increasing age, the median serum cysteine concentration increased, from 188 nmol/mL (IQR: 168–204 nmol/mL) in the <50 year old category to 203 nmol/mL (IQR: 186–225 nmol/mL) and 212 nmol/mL (IQR: 193–231 nmol/mL) in the 50–60 and > 60 year old categories. The median cysteine concentrations for OSCC and GCA cases were 191 nmol/mL (IQR: 172–211 nmol/mL) and 198 nmol/mL (IQR: 179–215 nmol/mL), respectively.

The paucity of data regarding the predictors of serum cysteine concentrations generally, (in any population) led us to investigate such factors in this population (Table 3). Age and BMI were significantly positively related and serum creatinine level was significantly inversely related to serum cysteine concentration.

Serum cysteine was significantly inversely associated with risk of both OSCC (HR: 0.86; 95% CI: 0.79, 0.93) and GCA (HR: 0.81; 95% CI: 0.73, 0.90) after adjusting for age, sex, cigarette smoking, alcohol drinking, BMI, and serum creatinine (Table 4). In quartile analysis, risk of both OSCC and GCA decreased significantly from the lowest to the highest quartile of serum cysteine ($P_{\text{trend}} = 0.006$ and $P_{\text{trend}} = 0.008$, respectively). Those in the top two quartiles of serum cysteine had a significantly lower risk of OSCC, relative to those in the lowest quartile (HR: 0.61, 95% CI: 0.44, 0.86 and HR: 0.70, 95% CI: 0.51, 0.98 for quartiles 3 and 4, respectively). Those in the highest quartile of serum cysteine also had a significantly lower risk of GCA, relative to those in the lowest quartile (HR: 0.59, 95% CI: 0.38, 0.91).

Adjusted hazard ratios for risk of OSCC and GCA stratified by sex, age, cigarette smoking and alcohol drinking are presented in Table 5. Significant interactions were noted between age and serum cysteine concentration for both OSCC ($P = 0.00014$) and GCA ($P < 0.0001$); the inverse association between serum cysteine concentration and both tumours was stronger among older people.

Discussion

In this large prospective cohort, we found that higher serum cysteine concentrations were associated with significantly reduced risk of both OSCC and GCA. Those in the highest quartile of serum cysteine had a 30% lower risk of OSCC and a 40% lower risk of developing GCA relative to those in the lowest quartile. These inverse associations were stronger for those in the older age groups for both cancer sites. Of note, the serum cysteine concentrations reported here (median: 196 nmol/mL; IQR: 178–216 nmol/mL) were significantly lower than those reported for healthy/control subjects in the UK (median: 240 nmol/L; IQR: 216–262 nmol/L) [24] and Norway (median: 318 nmol/L; IQR: 289–337 nmol/L) [25].

This is the first study to evaluate the relationship between serum cysteine concentration and risk of oesophageal or gastric cancer. Indeed, we could identify just 3 studies that have looked at (plasma) cysteine in the context of malignancies at other sites. Most recently, one study examined the association between cysteine, homocysteine, and cancer risk in 812 invasive breast cancer cases and matched controls [14]. This group reported a positive association between cysteine and breast cancer risk; those in the highest quintile of plasma cysteine had a relative risk of 1.65 (95% CI: 1.04, 2.61) compared to those in the lowest quintile, and the association was dose-dependent ($P_{\text{trend}} = 0.04$). In contrast, an investigation of 712 incident *in situ* and invasive breast cancer cases found that women in the highest quintile of plasma cysteine had a significantly lower risk of breast cancer relative to those in the lowest quintile (RR: 0.44; 95% CI: 0.26, 0.74), and this inverse association was dose-dependent ($P_{\text{trend}} = 0.002$) [15]. Lastly, a study of 147 women with squamous intraepithelial lesions of the cervix, 185 women with atypical squamous cells of undetermined significance (ASCUS), and 191 women with cytologically normal Pap smears reported that women in the highest quartile of plasma cysteine had a significantly lower risk of ASCUS than those in the lowest quartile (OR: 0.3; 95% CI: 0.2, 0.7) [13]. Given the large number of biologic pathways which cysteine is known to modulate, either directly or indirectly, it may not be surprising that it has been both positively and inversely associated with cancer risk in previous studies: its role in carcinogenesis is likely determined by both tumour type and physiologic context.

While our results suggest some potential for cysteine (or the related *N*-acetylcysteine) as a chemopreventive agent, evidence from the few reported animal and human studies is mixed. In a rat model where oesophageal papillomas and squamous cell carcinomas and liver tumours were induced, oral administration of *N*-acetylcysteine significantly reduced the multiplicity of oesophageal tumours but had no effect on the incidence or multiplicity of liver tumours [26]. A second study, using a rat model of oesophageal adenocarcinoma where tumours were induced via oesophagogastrroduodenal anastomosis, reported that *N*-acetylcysteine alone did not significantly decrease the incidence of oesophageal adenocarcinomas; however *N*-acetylcysteine in combination with α -tocopherol produced a significant reduction in tumour incidence [27]. Further evidence from animal studies suggests that *N*-acetylcysteine dose may be critical in determining its effect. In a rat mammary cancer model, a low dose of *N*-acetylcysteine modestly decreased, and a high dose significantly increased, tumour occurrence [28]. The single study that we have identified which related to gastric cancer reported that *N*-acetylcysteine inhibited human gastric cancer SJ-89 cell growth by inducing apoptosis and DNA synthesis arrest [29]. A randomized trial of 2592 former or current smokers with head and neck or lung cancers found no effect on survival or the incidence of second primary tumours following 2 years of *N*-acetylcysteine supplementation [30]. On the other hand, a small study of 7 volunteers found that carcinogenic acetaldehyde produced by smoking cigarettes could be completely inactivated by sucking a tablet containing 5mg of L-cysteine [4], suggesting some potential for cysteine as a chemopreventive agent in certain settings.

Without accurate assessment of dietary intake, it is impossible to tell whether serum cysteine may correlate with general protein intake in the current study population. In another analysis from the same cohort, individuals eating meat more than 12 times a year had a significantly reduced risk of OSCC (relative to those who ate meat less than 4 times a year). Higher egg consumption (>36 times/year, relative to 2 or fewer times/year) was associated with a significantly reduced risk of both OSCC and GCA [31]. However, it is likely that higher intake of protein (namely meat and eggs) is a proxy for both higher socioeconomic status and higher general nutritional status.

Our study has a number of strengths, including the prospective study design, the large number of cancer cases, the availability of data on potential confounders, a state-of-the-art measurement method for serum cysteine concentrations and the virtually complete follow-up of all study participants.

Our study also has several limitations. As with any study of this kind, unidentified, unmeasured confounders could explain the association we report (for Table 3 the total model $r^2 = 0.18$). The large size of the study, the precision of the lab analyses, the close follow-up of trial participants, and the rigorous documentation of cancer diagnoses suggest that neither exposure nor disease misclassification are likely to have significantly influenced our estimates. There is always a concern that pre-clinical disease could lead to alterations in serum measurements, thus creating a misleading association, but we found no difference in the exposure-disease associations between those with cancers diagnosed within the first 2 years of the study and those diagnosed after 2 years. Cysteine participates in a myriad of biological pathways and the specific pathways involved in the risk reduction we observed are not clear. Lastly, the generalisability of our study may be limited, due to the specificities of the population under study (see Table 1). In particular, low protein intake in this population [32] may be associated with the relatively low range of serum cysteine concentrations which we found.

In this large prospective population study, we found that individuals with higher baseline serum concentrations of cysteine were associated with a significantly reduced risk of both OSCC and GCA. These associations appear biologically plausible. Cysteine should be investigated for its potential as a chemopreventive agent for upper gastrointestinal cancers.

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Abbreviations

OSCC	oesophageal squamous cell carcinoma
GCA	gastric cardia cancer

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Summary Box

What is already known about this subject?

- Cancers of the stomach and oesophagus remain a significant cause of morbidity and mortality.
- Cysteine is a non-essential amino acid involved in immuno-modulatory, anti-oxidant, and anti-carcinogenic pathways.
- Though previously proposed as a chemopreventive agent, epidemiological studies of cysteine have produced equivocal results.

What are the new findings?

- In this large prospective cohort, higher serum cysteine concentrations are associated with a 30% lower risk of oesophageal squamous cell carcinoma and a 40% lower risk of gastric cardia adenocarcinoma.
- The inverse associations between serum cysteine and both oesophageal and gastric cancers are dose dependent.

How might it impact clinical practice in the foreseeable future?

- Cysteine warrants further investigation regarding its potential as a chemopreventive agent for upper gastrointestinal cancers.

Table 1

Study characteristics of cases and the sub-cohort from the Linxian General Population Nutrition Intervention Trial cohort, overall and by sex.

Variable	Sub-cohort*	Oesophageal Squamous Cell Carcinoma	Gastric Cardia Adenocarcinoma
Number, Total N (%)	947	498	255
Men	515 (54%)	241 (48%)	167 (65%)
Women	432 (46%)	257 (53%)	88 (35%)
Age at baseline, Mean (SD)	57 (7.7)	57 (7.8)	58 (6.4)
Men	59 (6.8)	58 (7.0)	58 (6.1)
Women	56 (8.4)	55 (8.3)	56 (6.7)
Cigarette Smoking, N (%) yes[‡]	364 (38%)	190 (38%)	118 (46%)
Men	362 (70%)	190 (79%)	118 (71%)
Women	2 (<1%)	0 (0%)	0 (0%)
Alcohol Drinking, N (%) yes[§]	200 (21%)	112 (23%)	58 (23%)
Men	172 (33%)	95 (39%)	51 (31%)
Women	28 (6%)	17 (7%)	7 (8%)
BMI (Kg/m²), Mean (SD)	21.8 (2.6)	21.4 (2.4)	21.6 (2.5)
Men	21.5 (2.1)	21.6 (2.1)	21.3 (2.0)
Women	22.1 (3.1)	21.3 (2.6)	22.1 (3.2)
Serum Creatinine (mg/dL), Mean (SD)	0.84 (0.18)	0.83 (0.20)	0.86 (0.22)
Men	0.91 (0.15)	0.92 (0.16)	0.93 (0.16)
Women	0.75 (0.17)	0.75 (0.19)	0.72 (0.24)

* Some subjects who later developed one of these cancers are included in the randomly selected sub-cohort.

[‡] Daily cigarette consumption among men was generally low and few subjects had quit smoking, so smoking is presented as never smoking versus ever smoking.

[§] Alcohol consumption was minimal at the time of the baseline interview (1985), so alcohol drinking was categorized as any consumption in the previous twelve months versus none

Table 2

Serum cysteine concentration (nmol/mL) quantiles in the sub-cohort from the Linxian General Population Nutrition Intervention Trial cohort, overall and by sex, age, smoking, and drinking status.

	Quantile		
	25 th	50 th	75 th
General Population Trial Cohort*			
Overall	178	196	216
Sex			
Men	179	196	215
Women	176	196	217
Age (in years)			
<50	168	188	204
50–60	186	203	225
>60	193	212	231
Cigarette Smoking[‡]			
Never smokers	181	194	219
Ever smokers [‡]	179	196	212
Alcohol Drinking[§]			
Non-Drinkers	174	195	216
Drinkers [§]	183	200	213

* All concentrations are weighted by the age and sex sampling to reflect the distributions in the full cohort

[‡]Daily cigarette consumption among men was generally low and few subjects had quit smoking, so smoking is presented as never smoking versus ever smoking.

[§]Alcohol consumption was minimal at the time of the baseline interview (1985), so alcohol drinking was categorized as any consumption in the previous twelve months versus none

Table 3

Predictors of serum cysteine concentration in the sub-cohort from the Linxian General Population Nutrition Intervention Trial cohort, from multivariable linear regression models.

Variable	β coefficient	Standard error	P
Intercept	242.41	12.063	<.0001
Sex, male	-0.020	2.72	0.99
Age [*] , 1 year	1.38	0.1001	<.0001
Cigarette Smoking, ever	-4.51	2.76	0.10
Alcohol Drinking, any	3.54	2.30	0.13
BMI [†] , 1 kg/m ²	1.79	0.35	<.0001
Creatinine, 0.12 units	-13.03	3.78	0.0006
Creatinine squared	1.019	0.29	0.0005

* Age was centred at 55 years old in the model, the middle of the sub-cohort age distribution

[†]Body mass index (BMI) was centred at 22 units, the sub-cohort median

Hazard ratios (HRs) and 95% confidence intervals (CIs) for the association between serum cysteine concentration and risk of oesophageal squamous cell carcinoma and gastric cardia adenocarcinoma in the Linxian General Population Nutrition Intervention Trial cohort.

Table 4

Group	Continuous [‡]		Quartile					
			Q1	Q2		Q3	Q4	
	HR	95% CI	P	HR	95% CI	HR	95% CI	P _{trend} [§]
Oesophageal Squamous Cell Carcinoma								
Age & sex-adjusted	0.87	0.81, 0.94	0.00027	1.00	1.01	0.77, 1.34	0.70	0.52, 0.93
Fully adjusted *	0.86	0.79, 0.93	0.00016	1.00	0.96	0.69, 1.34	0.61	0.44, 0.86
Gastric Cardia Adenocarcinoma								
Age & sex-adjusted	0.85	0.76, 0.94	0.00093	1.00	1.06	0.70, 1.62	0.96	0.64, 1.45
Fully adjusted *	0.81	0.73, 0.90	<0.0001	1.00	0.95	0.61, 1.48	0.77	0.50, 1.19
								0.46, 1.06
								0.38, 0.91
								0.0081

* Fully adjusted HRs and 95% CIs in were calculated using models stratified on age and sex, with additional adjustment for continuous age variables within each stratum, cigarette smoking, alcohol drinking, BMI, serum creatinine and serum creatinine squared

[‡]Continuous HRs are scaled to 19 nmol/mL, which is one half of the inter-quartile range in the population distribution (75th percentile – 25th percentile ÷ 2)

[§]P for trend comes from a model where quartile of cysteine was entered as an ordinal variable

Table 5

Adjusted* hazard ratios (HR) and 95% confidence intervals (CI) for the association between serum cysteine concentration and risk of oesophageal squamous cell carcinoma and gastric cardia adenocarcinoma in the Linxian General Population Nutrition Intervention Trial cohort, stratified by sex, age, cigarette smoking and alcohol drinking.

	Oesophageal Squamous Cell Carcinoma			Gastric Cardia Adenocarcinoma		
	HR	95% CI	P interaction [‡]	HR	95% CI	P interaction [‡]
Overall	0.86	0.79, 0.93		0.81	0.73, 0.89	
Sex						
Men	0.89	0.80, 0.98		0.82	0.73, 0.93	
Women	0.82	0.72, 0.92	0.19	0.77	0.64, 0.94	0.66
Age (in years)						
(<50)	0.92	0.74, 1.14		1.10	0.76, 1.58	
(50-60)	0.89	0.80, 1.00		0.79	0.69, 0.91	
(>60)	0.80	0.70, 0.91	0.0014	0.79	0.66, 0.94	<0.0001
Cigarette smoking [‡]						
Never smokers	0.91	0.73, 1.13		0.79	0.62, 1.00	
Ever smokers	0.89	0.79, 1.00	0.89	0.84	0.73, 0.97	0.41
Alcohol drinking [‡]						
Non-drinkers	0.87	0.76, 1.00		0.77	0.66, 0.90	
Drinkers	0.93	0.78, 1.10	0.94	0.86	0.69, 1.07	0.30

* Adjusted HRs and 95% CIs in were calculated using models stratified on age and sex, with additional adjustment for continuous age variables within each stratum, cigarette smoking, alcohol drinking, BMI, serum creatinine and serum creatinine squared

[‡] Cigarette and alcohol consumption were almost completely restricted to men, so data stratified by these exposures are not presented for women