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Plasma and dietary vitamin C levels and risk of gastric cancer in the European Prospective Investigation into Cancer and Nutrition (EPIC-EURGAST)

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Vitamin C is an antioxidant and inhibitor of carcinogenic *N*-nitroso compound production in the stomach. Higher dietary vitamin C consumption is associated with decreased risk of gastric cancer (GC) in numerous case–control studies, but data from prospective studies are limited, particularly so for blood measures of vitamin C. The objective of this study was to determine the association of plasma and dietary vitamin C levels with the risk of GC in a case–control study nested within the European Prospective Investigation into Cancer and Nutrition (EPIC), a large cohort involving 10 European countries. Using a fluorometric method, vitamin C was measured in pre-diagnostic plasma from 215 GC cases (matched controls = 416). Conditional logistic regression models adjusted by body mass index, total energy intake, smoking status/duration/intensity and *Helicobacter pylori* infection status were used to estimate relative cancer risks. No association with GC risk was observed for dietary vitamin C, whereas an inverse GC risk was observed in the highest versus lowest quartile of plasma vitamin C [odds ratio (OR) = 0.55, 95% confidence interval (CI) = 0.31–0.97, P_{trend} = 0.043], which was maintained after exclusion of cases with ≤2 years follow-up (OR = 0.40, 95% CI = 0.19–0.83, P_{trend} = 0.064). The inverse association was more pronounced in subjects consuming higher levels of red and processed meats, a factor that may increase endogenous *N*-nitroso compound production. The effect of plasma vitamin C was not different by GC anatomical subsite (cardia/non-cardia) or histological subtype (diffuse/intestinal), and there was no significant interaction of effect with *H.pylori*. The results of this study show, in a prospective setting, an inverse association of GC risk with high levels of plasma vitamin C and suggest an interaction with the intake of red and processed meats, whose consumption may elevate endogenous *N*-nitroso compound production.

Introduction

In many Western countries, the relative incidence rates for adenocarcinomas of the gastric cardia have increased (1,2). Several environmental factors, particularly diet, are thought to be involved in the etiology of gastric cancers (GC). Out of the many dietary components that have been associated with GC risk, antioxidants tend to show the strongest protective effects, and by far the most effective of these is vitamin C (3). In fact, the consumption of citrus fruits, which are rich

Abbreviations: EPIC, European Prospective Investigation into Cancer and Nutrition; EU, ELISA units; GC, gastric cancer; GEJ, gastro-esophageal junction.

dietary sources of vitamin C, has been shown to be inversely associated with GC risk (3–5).

Vitamin C is an important enzyme co-factor (6) and has also been suggested to have anti-proliferative and pro-apoptotic roles *in vitro* (6), to inhibit the growth of *Helicobacter pylori* (7) and to regulate the immune response towards *H.pylori* infection (8). But perhaps most importantly, vitamin C can quench reactive oxygen species produced in the gastric environment, thus limiting free radical-mediated damage in the gastric epithelium (9), and it can scavenge nitrite, inhibiting *in vivo* nitrosation and the production of carcinogenic *N*-nitroso compounds (10). Normally, gastric mucosa and juice contain high levels of vitamin C, perhaps even higher levels than in plasma (11). But in the presence of gastric pathology, the vitamin C secretion into the gastric juice is affected, causing lower vitamin C concentrations (12). GC patients have been shown to have decreased levels of blood vitamin C, increased pH and nitrite in their gastric juice (13) and higher overall levels of oxidative stress (14). In fact, low dietary vitamin C intake can enhance the progression of gastric dysplasia to GC (15), whereas vitamin C supplementation can slow the progression of gastric mucosal atrophy (16) and increase the regression rate of gastric pre-cancerous lesions (17).

Many case–control studies show an inverse association between dietary vitamin C intake and GC risk (3,4,18,19). Although the effect of diet on GC risk may differ on the basis of the subsite localization within the stomach or histological subtype of the cancer (20,21), few previous studies have considered these factors. In recent case–control studies, dietary vitamin C has been shown either to have no association with cardia GC (22–24) or to be inversely associated with both GC subsites (25,26) and subtypes (25,27). Much of this disparity in results may be due to the inherent measurement errors in dietary recall instruments and biases associated with case–control study designs. In the few existing data from prospective studies, both increased dietary (28–30) and blood (15,31,32) vitamin C levels have been associated with a decreased GC risk.

Thus, the aim of this case–control study, nested within the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort, was to determine the association of plasma and dietary vitamin C levels with risk of GC (as well as its subsites and subtypes), taking into account *H.pylori* infection status.

Materials and methods

Study population and collection of blood samples

The rationale and methods of the EPIC study have been previously discussed in detail (33,34). Briefly, EPIC consists of 23 centers in 10 European countries (Denmark, France, Greece, Germany, Italy, Netherlands, Norway, Spain, Sweden and United Kingdom). Between 1992 and 1998, standardized lifestyle and personal history questionnaires, anthropometric data and blood samples were collected from most participants. In addition, diet over the previous 12 months was measured at recruitment by validated country-specific questionnaires designed to ensure high compliance and better measures of local dietary habits (33,34). Values for total energy and dietary vitamin C were computed using country-specific food composition tables. Data on the intake of vitamin C from dietary supplements are not available and were not assessed here.

As has been previously documented (33,34), in each of the 23 centers, blood samples of at least 20 ml were drawn from all participants and stored at 5–10°C, protected from light and transported to local laboratories for processing and aliquoting. The only exceptions were the EPIC-Oxford center (UK) where blood samples were collected from a network of general practitioners and transported to a central laboratory in Norfolk via mail, and centers in Sweden where blood was aliquoted within 1 h of drawing.

In all countries, except Sweden, blood was separated into 0.5 ml fractions (serum, plasma, red cells and buffy coat for DNA extraction) and stored in heat-sealed straws at ultra-low temperatures (−196°C) under liquid nitrogen. One half of all aliquots were stored at the local study center and the other half in the central EPIC biorepository at the International Agency for Research on Cancer (IARC; Lyon, France). In Sweden, samples were stored in −80°C freezers.

Short-term losses of blood vitamin C from handling, transport and storage prior to long-term freezing have previously been shown to be minimal (35). Before long-term freezing, blood samples were not treated with compounds, such as meta-phosphoric acid, for the specific stabilization of vitamin C. Regardless, a recent study specifically on EPIC plasma samples shows that after freezing at −196°C for up to 11 years, vitamin C can still be measured with reasonable reliability as a biomarker (36).

Follow-up for cancer incidence and vital status

Follow-up is based on population cancer registries (Denmark, Italy, Netherlands, Norway, Spain, Sweden and the United Kingdom) and other methods, such as health insurance records, pathology registries and active contact of study subjects or next of kin (France, Germany and Greece). The follow-up period for the present study was for data reports received at IARC to the end of October 2002, representing complete follow-ups until either December 2000 or December 2001 for all centers using cancer registry data and until 2002 for France, Germany and Greece. Cancers of the stomach included cancers coded as C16 (10th Revision of the International Statistical Classification of Diseases, Injury and Causes of Death). The diagnosis, tumor site classification and morphology (according to ICDO2 and Lauren classifications) of each identified cancer was confirmed and validated by an independent panel of pathologists with a representative from each EPIC country and a coordinator. The pathologist panel reviewed histological slides and/or re-cuts from the paraffin blocks and original histopathology reports provided by each EPIC center.

Nested case–control study design and selection of study subjects

The present study includes all GC incident cases (excluding gastric lymphomas, gastric stump cancers, other gastric non-adenocarcinoma, esophageal non-adenocarcinomas and otherwise unspecified malignant neoplasms) with available blood samples that were diagnosed after recruitment from all EPIC countries except Norway (blood samples only recently collected). Out of a total of 230 gastric adenocarcinomas and 18 adenocarcinomas of the gastro-esophageal junction (GEJ) cases identified, plasma samples for 33 were of insufficient volume/quality for vitamin C analysis. Thus, the present study includes a total of 199 gastric adenocarcinomas and 16 GEJ, which are grouped together (matched controls, $n = 416$) and referred to as GC. GC were also divided into three groups by anatomical subsite: (i) cardia tumors (cases, $n = 59$; matched controls, $n = 113$), combining tumors that reached the GEJ, either crossing it from below (all 16 GEJ adenocarcinomas) or not; (ii) non-cardia tumors (cases, $n = 113$; matched controls, $n = 223$), grouping cases from other sites in the stomach; and (iii) tumors from unknown or mixed sites (cases, $n = 43$; matched controls, $n = 80$). When divided by histological subtype, of the 215 GC cases, 86 were classified as diffuse (matched controls, $n = 166$) and 83 as intestinal (matched controls, $n = 163$) histological subtypes according to the Lauren classification. The remainder (cases, $n = 46$; matched controls, $n = 87$) were of unknown or mixed histological types. For each identified GC case, control subjects with available blood samples were selected from all cohort members who were alive and free of cancer (except non-melanoma skin cancer) at the time of diagnosis of the case patient. Controls were matched (1:2) by gender, age group (±2.5 years), study center and date of blood sample collection (±45 days; to account for follow-up time and seasonality). Plasma vitamin C measurements could not be obtained for 14 control subjects and so they were excluded.

Laboratory assay—H.pylori infection status

The methodology for the determination of *H.pylori* infection status is detailed elsewhere (5,37). Briefly, quantification of anti-*H.pylori* antibodies in plasma of all cases and controls was done by enzyme-linked immunosorbent assay (ELISA) using the lysate of the *H.pylori* CCUG strain. Briefly, various dilutions of plasma samples (starting dilution 1:200) were incubated with the *H.pylori* lysate in solid phase (1 ug/ml). After 1 h and extensive washings, plates were incubated with an alkaline phosphatase-conjugated polyclonal affinity purified goat anti-human IgG (Sigma chemical, St Louis, MO). After 3 h incubation and further washings, the enzymatic reaction was revealed by addition of *p*-nitrophenylphosphate as a

substrate. *H.pylori*-specific IgG antibody titres were expressed as ELISA units (EU), and were determined by interpolation relative to a standard curve constructed by a serial dilution of a standard positive control. A cut-off value of 100 EU was defined using serum samples from individuals negative for *H.pylori* infection as determined by clinical, microbiological and serological assays (western blotting). Serum samples with EU values >100 were considered as positive for anti-*H.pylori* IgG antibodies. In previous experiments this assay exhibited specificity and sensitivity > 90%.

Laboratory assay—vitamin C

Vitamin C measurements were performed at Addenbrookes Hospital (Cambridge, UK) using a fluorometric analysis method, described in detail previously (38). Plasma from blood initially drawn into citrate tubes was removed from storage, thawed and then stabilized with a standardized volume of freshly prepared meta-phosphoric acid. Samples were quickly refrozen to −70°C and shipped on dry ice to Addenbrookes Hospital for analysis. All samples were run in duplicate with matched case–control sets assayed in the same batch in order to minimize errors from batch to batch variations. Low (coefficient of variation = 14.0%) and high (coefficient of variation = 7.4%) concentration vitamin C quality control samples were run at the beginning and end of each analysis batch. Samples with >10% difference between duplicates were repeated. Laboratory technicians were blinded to the case–control status of all samples. No significant between-day drift was observed.

Statistical methods

Differences between cases and controls in mean vitamin C levels and baseline covariates were tested by paired *t*-tests. The correlation between dietary and plasma vitamin C was assessed by way of a Spearman Rank Correlation Test, adjusted for age, smoking duration/status, body mass index, total energy intake and *H.pylori* positivity.

Odds Ratios (OR) and 95% confidence intervals (95% CI) for GC in relation to plasma and dietary vitamin C concentrations were calculated by conditional logistic regression using the PHREG procedure (SAS statistical software, version 9, SAS Institute, Cary, NC), stratified by the case–control set. Risk estimates were computed both as 'crude' (adjustment for matching variables only) and as 'fully adjusted' with additional adjustments for potential confounders including total energy intake (in quartiles), body mass index (quartiles), *H.pylori* infection status (yes/no) and duration/status/intensity of smoking (variable categories: never smokers, ex-smokers who smoked for <10 years, ex-smokers who smoked for ≥10 years, smokers who smoke <15 cigarettes/day, smokers who smoke between 15–25 cigarettes/day, smokers who smoke ≥25 cigarettes/day, and missing). The effects of alcohol intake and the level of schooling (an indicator variable for socioeconomic status) as potential confounding variables were examined, but they did not provide appreciable changes in risk estimates and were not included in the models. For both plasma and dietary vitamin C, risk associations were examined by quartiles with cut points based on the distribution of vitamin C in the GC control subjects. Models similar to the above were also run with plasma and dietary vitamin C measurements included in the model as continuous variables, with the ORs estimated for the risk related to a change in the plasma or dietary level by 1 SD of the distribution in control subjects (19.4 μmol/l for plasma and 72.9 mg/day for dietary vitamin C). For all models, tests for linear trend were performed using a score variable with values from 1 to 4, consistent with the quartile grouping.

The models for plasma vitamin C were run separately for GC, as well as by anatomical subsite (cardia/non-cardia) and histological subtype (diffuse/intestinal). The same quartile cut points used for analysis of all GCs were used in subgroup analyses. Tests for heterogeneity were also run for comparison of results by subsite and subtype. As described above, for a number of reasons, some GC cases could not be classified by anatomical subsite or histological subtype. For comparison purposes, ORs were also calculated for these cases and matched controls, using the same methods described above.

Potential modification of the effects of vitamin C by gender, *H.pylori* infection status and time of follow-up of ≤2 years or >2 years was tested using the likelihood ratio test to assess the statistical significance of a linear interaction. For the assessment of interaction for time of follow-up, each case–control set was assigned the value for years of follow-up of the case. No overall significant interactions were observed for any of these variables. In order to assess the effect of time of follow-up in greater detail, analyses were also performed for plasma vitamin C and GC risk, excluding cases diagnosed with <2 years of follow-up.

H.pylori infection status was used as a confounding variable because of its purported association with GC risk and its potential to alter the systemic bioavailability of vitamin C (39) and the vitamin C concentration of gastric juice (40,41). To further explore the role of *H.pylori* infection status, unmatched case–control analyses stratified by this variable using plasma vitamin C modeled as a continuous variable were performed by using unconditional logistic regression models adjusted for case–control matching variables, as well as the laboratory batch and all other variables described in the 'fully adjusted' model above. In order to explore any role of smoking, a similar model to the above was used, stratified by smoking status (never, former, current, with missing in a separate category).

In order to correct for measurement errors in dietary vitamin C assessment, a linear calibration model was employed (42). For this purpose, dietary vitamin C values derived from standardized 24 h dietary recall measurements collected at baseline from an 8% subset of the EPIC cohort were taken as reference measurements (43). They were linearly regressed on questionnaire measures of dietary vitamin C intake in order to compute a set of predicted values for all subjects (44). The predicted values were used in the risk model to evaluate a corrected association between dietary vitamin C and GC on a continuous scale. The calibration model included center-specific terms, as well as a list of confounding variables identical to the 'fully adjusted' model described above. A bootstrap sampling procedure with 300 repetitions was employed to compute the standard error of the corrected coefficient.

Since low levels of vitamin C and higher intake of red and processed meats are factors that may increase intra-gastric *N*-nitroso compound production, a potential interaction of effect of plasma vitamin C with dietary intake of red and processed meats was explored by modeling a smoothed dose–response relationship using the exposures and their interaction as continuous variables. The statistical significance of a linear interaction was assessed using the likelihood ratio test. ORs were computed for different levels of plasma vitamin C and dietary red and processed meat intake, and the associated 95% CI were assessed through a bootstrap sampling procedure with 1000 repetitions (45). The reference category was set as low level of plasma vitamin C and high intake of red and processed meats. In theory, this category would be expected to show the highest production of endogenous *N*-nitroso compounds in this population.

Results

Description of the study population

Table I shows the baseline characteristics and description of the study population. The mean age at recruitment of GC cases was (±SD) 59.3 ± 8.2 and controls was 59.4 ± 8.2 (Table I). On average, GC cases had 3.3 years between blood donation and diagnosis. GC cases had a higher percentage of

Table I. Baseline characteristics and description of the study population

GC	Cases (n = 215)	Controls (n = 416)
Age at recruitment	59.3 ± 8.2	59.4 ± 8.2
Age at diagnosis	62.7 ± 8.6	NA
Mean number of years between blood donation and diagnosis	3.3 ± 2.2	NA
Percent *H.pylori* positive	86.5	70.9
Body mass index	26.5 ± 3.9	26.5 ± 4.2
Number of males	119	230
Number of females	96	186
Grouping by anatomical subsite:		
Cardia, no. of subjects	59	113
Non-cardia, no. of subjects	113	223
Unknown or mixed subsite, no. of subjects	43	80
Grouping by histological subtype:		
Diffuse, no. of subjects	86	166
Intestinal, no. of subjects	83	163
Unknown or mixed subtype, no. of subjects	46	87

Values are either means ± standard deviation, or number of subjects, except for *H.pylori* positivity, which is given as an overall percentage, as indicated. For GCs, distribution of cases/controls by country: Francec = 3/6, Germany = 30/60, Greece = 12/23, Italy = 43/84, Netherlands = 18/35, Spain = 27/52, Sweden = 54/102 and United Kingdom = 28/54.

H.pylori positivity than controls, while body mass index was similar between cases and controls (Table I).

Plasma vitamin C

Table II shows the mean plasma vitamin C values and standard deviations in cases and controls, as well as the *P*-value for difference between cases and controls for all the GC groupings. For GC, the mean plasma vitamin C (±SD) was 39.9 ± 25.2 μmol/l in cases and 41.4 ± 19.4 μmol/l in controls ($P_{difference} = 0.26$). Plasma vitamin C values of cases and controls did not differ by the follow-up period (data not shown).

The association of plasma vitamin C with GC risk showed an inverse association that was significant in the highest versus the lowest quartile in both the crude (OR = 0.53, 95% CI = 0.31–0.90, $P_{trend} = 0.023$) and fully adjusted models (OR = 0.55, 95% CI = 0.31–0.97, $P_{trend} = 0.043$) (Table III). A significant negative association was maintained after the exclusion of cases with <2 years of follow-up (OR of the highest versus the lowest quartile = 0.40, 95% CI = 0.19–0.83, $P_{trend} = 0.064$; fully adjusted model).

The smoothed dose–response analysis of the interaction between plasma vitamin C and intake of red and processed meats showed that the decrease in GC risk with increasing plasma vitamin C concentration was more apparent in subjects with medium and high levels of red and processed meat intake (Table IV). The *P*-value for interaction between plasma vitamin C levels and intake of red and processed meats was 0.058.

H.pylori infection status

Plasma vitamin C values of the controls were not significantly different between *H.pylori* positive (42.1 ± 1.1 μmol/l) and *H.pylori* negative (39.4 ± 1.8 μmol/l). The GC risk association of plasma vitamin C based on *H.pylori* infection status was explored via unconditional logistic regression models with vitamin C modeled as a continuous variable. In the fully adjusted model, the ORs were estimated for a 19.4 μmol/l increment (*H.pylori* negative = 1.23, 95% CI = 0.67–2.27; *H.pylori* positive = 0.89, 95% CI = 0.74–1.08).

Smoking status

Plasma vitamin C values of the controls were not significantly different by smoking status, although values in never smokers (43.5 ± 1.1 μmol/l; mean ± standard error) were slightly higher than former smokers (40.6 ± 1.7 μmol/l) and current smokers (38.9 ± 2.0 μmol/l). The GC risk association of plasma vitamin C stratified by smoking status was explored via unconditional logistic regression models with vitamin C modeled as a continuous variable, and ORs were calculated for a 19.4 μmol/l increment (never smokers = 1.13, 95% CI = 0.86–1.47; former smokers = 0.91, 95% CI = 0.67–1.24; current smokers = 0.67, 95% CI = 0.43–1.06).

Dietary vitamin C

The mean dietary vitamin C (±SD) was 129.5 ± 81.6 mg/day for GC cases and 128.1 ± 72.9 mg/day for GC controls (Table II). Dietary and plasma vitamin C levels were equally correlated in both the GC control subjects ($r = 0.18$, $P < 0.001$) and cases ($r = 0.17$, $P = 0.016$). Dietary vitamin C showed no significant associations with GC risk at any level of intake (Table III). In the fully adjusted model, the OR for a 72.9 mg/day increase in dietary vitamin C intake was 1.09 (95% CI = 0.90–1.33). Using the calibrated predicted values for dietary vitamin C, the calibrated OR for a similar increment in daily intake was reduced to 0.95 (0.66–1.37).

Grouping by anatomical subsite and histological subtype

There were no significant differences in mean plasma vitamin C concentrations between cases and controls by GC subsite or subtype (Table II). Table III shows the ORs and CIs for quartiles of increasing plasma vitamin C values and risk of GCs by anatomical subsite and histological subtype. The *P*-value for heterogeneity between the cardia and non-cardia subsites was 0.129 for the crude model and 0.091 for the fully adjusted model. For the diffuse versus the intestinal subtype, the *P*-value for heterogeneity was 0.455 for the crude model and 0.659 for the fully adjusted model. No statistically significant associations were observed with risk of either cardia or non-cardia GCs at any quartiles of plasma vitamin C, although the direction of effect was always negative, particularly in the cardia. Similarly, no significant associations were observed with risk of either the diffuse or intestinal subtypes, at any category of plasma vitamin C. For comparison purposes, the ORs for groups of cases of unknown anatomical subsite (OR = 0.83, 95% CI = 0.51–1.32) or unknown/mixed histological subtype (OR = 0.76,

Table II. Means, standard deviation and distribution of plasma vitamin C levels amongst cases and controls in GCs and adenocarcinoma of the esophagus

	Cases		Controls		*P* for difference in mean vitamin C levels[a]	5th–95th percentile distribution of vitamin C[b]	
	No.	Mean vitamin C level ± SD	No.	Mean vitamin C level ± SD		Cases	Controls
GCs							
Plasma vitamin C (μmol/l)[a]	215	39.9 ± 25.2	416	41.5 ± 19.4	0.26	11.0–82.0	12.0–75.0
Dietary vitamin C (mg/day)[a]	215	129.5 ± 81.6	416	128.1 ± 72.9	0.67	41.0–279.1	42.7–248.7
Grouping of GCs by anatomical subsite—plasma vitamin C (μmol/l)							
Cardia GCs	59	37.0 ± 17.9	113	40.7 ± 18.3	0.08	10.0–79.0	15.0–77.0
Non-cardia GCs	113	42.5 ± 27.7	223	42.2 ± 20.6	0.88	14.0–88.0	12.0–76.0
Grouping of GCs by histological subtype—plasma vitamin C (μmol/l)							
Diffuse GCs	86	43.8 ± 31.7	166	44.6 ± 20.3	0.81	9.0–88.0	17.0–86.0
Intestinal GCs	83	37.9 ± 20.0	163	40.6 ± 18.7	0.25	10.0–82.0	12.0–67.0

[a]Two-sided *P*-values, paired *t*-test, given for a difference between cases and controls.
[b]Values represent the lowest and highest plasma vitamin C values of all controls in each cancer grouping, in the 5th and 95th percentiles, respectively.

Table III. ORs for quartiles of increasing levels of plasma and dietary vitamin C and risk of GCs

GC	OR for quartiles of plasma or dietary vitamin C levels[a]				*P*-trend[b]	OR of 1 SD increase[c]
	Ref.	2	3	4		
Plasma vitamin C	<29.0	≥29.0–<40.0	≥40.0–<51.0	≥51.0 μmol/l		19.4 μmol/l
Cases/controls	70/101	46/98	54/112	45/105		
Mean (μmol/l)[d]	19.1 ± 0.7	34.1 ± 0.3	44.9 ± 0.3	66.3 ± 1.6		
Crude	1.00	0.64 (0.40–1.03)	0.67 (0.42–1.05)	0.53 (0.31–0.90)	0.023	0.90 (0.75–1.08)
Fully adjusted	1.00	0.73 (0.43–1.22)	0.70 (0.43–1.14)	0.55 (0.31–0.97)	0.043	0.93 (0.77–1.12)
Diet vitamin C	<78.0	≥78.0–<111.5	≥111.5–<160.0	≥160.0 mg/day		72.9 mg/day
Cases/controls	61/104	45/104	56/101	53/107		
Mean (mg/day)[d]	55.8 ± 1.5	96.9 ± 1.0	134.7 ± 1.4	223.3 ± 6.9		
Crude	1.00	0.75 (0.46–1.21)	0.94 (0.59–1.49)	0.85 (0.53–1.38)	0.719	1.02 (0.86–1.22)
Fully adjusted	1.00	0.77 (0.45–1.30)	0.94 (0.57–1.58)	1.02 (0.60–1.74)	0.769	1.09 (0.90–1.33)
Grouping of GCs by anatomical subsite						
Plasma vitamin C	<29.0	≥29.0–<40.0	≥40.0–<51.0	≥51.0 μmol/l		19.4 μmol/l
Cardia						
Cases/controls	17/29	16/28	16/29	10/27		
Crude	1.00	0.84 (0.35–2.01)	0.79 (0.32–1.96)	0.47 (0.16–1.40)	0.209	0.71 (0.47–1.07)
Fully adjusted	1.00	0.74 (0.25–2.16)	0.59 (0.21–1.66)	0.36 (0.10–1.33)	0.118	0.65 (0.40–1.06)
Non-cardia						
Cases/controls	35/54	19/52	34/57	25/60		
Crude	1.00	0.53 (0.26–1.08)	0.91 (0.49–1.68)	0.57 (0.28–1.18)	0.334	1.02 (0.81–1.29)
Fully adjusted	1.00	0.64 (0.30–1.39)	1.01 (0.51–2.03)	0.63 (0.28–1.42)	0.520	1.06 (0.82–1.37)
Grouping of GCs by histological subtype						
Plasma vitamin C	<29.0	≥29.0–<40.0	≥40.0–<51.0	≥51.0 μmol/l		19.4 μmol/l
Diffuse						
Cases/controls	27/30	17/44	21/43	21/49		
Crude	1.00	0.42 (0.20–0.91)	0.55 (0.27–1.14)	0.43 (0.19–1.00)	0.081	0.97 (0.75–1.25)
Fully adjusted	1.00	0.50 (0.18–1.38)	0.65 (0.25–1.66)	0.36 (0.13–0.99)	0.091	0.96 (0.72–1.27)
Intestinal						
Cases/controls	26/42	22/32	19/51	16/38		
Crude	1.00	1.02 (0.49–2.12)	0.58 (0.28–1.22)	0.58 (0.24–1.40)	0.101	0.83 (0.61–1.13)
Fully adjusted	1.00	1.21 (0.53–2.75)	0.58 (0.26–1.32)	0.59 (0.20–1.73)	0.138	0.85 (0.59–1.21)

[a]Values are ORs and 95% CIs derived from models described above based on quartiles of plasma levels of vitamin C for GCs and GCs subgroup analyses, and quartiles of dietary vitamin C for GCs.
[b]*P* of χ^2-test for trend using a continuous variable with 1 df.
[c]Values are ORs (95% CIs), derived from models as described above, for a risk associated with an increment in vitamin C level equal to the standard deviation of the controls for GCs (plasma: 19.4 μmol/l; diet: 72.9 mg/day).
[d]Values are means ± standard error calculated on the basis of the control subjects in each quartile of the respective variable.

Table IV. OR for a smoothed dose–response analysis of the interaction of increasing levels of plasma vitamin C and dietary intake of red and processed meats

Plasma vitamin C (μmol/l)	Dietary red and processed meat intake level [OR (95% CI)][a]		
	High ≥95.0 (g/day)	Medium ≥55.6–<95.0 (g/day)	Low <55.6 (g/day)
Low <32.0	1.00	0.80 (0.54–1.11)	0.69 (0.36–1.18)
Medium ≥32.0–<46.0	0.79 (0.58–1.00)	0.75 (0.46–1.06)	0.72 (0.37–1.20)
High ≥46.0	0.58 (0.28–1.00)	0.69 (0.35–1.17)	0.76 (0.36–1.50)

[a]Values are ORs (95% CI) derived from models described above based on a smoothed dose–response analysis with tertiles of plasma vitamin C and dietary intake of red and processed meats. 95% CI were assessed using a bootstrap sampling procedure.

95% CI = 0.46–1.25) were calculated for a 19.4 μmol/l increment in plasma vitamin C.

Discussion

This nested case–control study is one of the largest prospective analyses of the association of plasma and dietary vitamin C levels with GC risk ever performed on Western European populations. The results show that within the EPIC cohort, higher plasma vitamin C level is associated with a decreased risk of GC, and does not appear to be limited to a particular GC anatomical subsite or histological subtype. In contrast, dietary vitamin C showed no significant association with GC risk, even after linear calibration.

Vitamin C may plausibly be involved in GC prevention by way of its potential to modulate cell growth kinetics (46), its purported antimicrobial activity against *H.pylori* (7,47) and its antioxidant properties (9). These mechanisms can all directly relate to GC risk (12–14), and a potential GC protective effect of higher dietary vitamin C intake has been observed in ecological (48,49) and case–control studies (3,18,19,25–27). Although the demonstrated effect of vitamin C in these studies is quite strong, dietary data from case–control studies are nonetheless affected by recall bias, while biochemical values may be affected by the presence of the disease. It is for these reasons that data from prospective studies, particularly those using blood biomarkers, are often valuable in adding to the scientific knowledge in a given area. However, in the case of the association of blood vitamin C levels and GC, information from prospective studies is scarce, but existing data do show a generally inverse risk association in select European (31) and high-risk Chinese

populations (15,32)—although none were stratified by subsite or subtype. In this regard, by way of its prospective design and use of both dietary and plasma vitamin C measures, the present study adds considerably to the degree of knowledge in the field.

Another key mechanism of vitamin C action is its inhibition of *N*-nitroso compound formation within the stomach (10,50). In the present study, a borderline statistically significant interaction was observed between plasma vitamin C and dietary red and processed meats, whose higher intake is suggested to increase endogenous *N*-nitroso compound formation (51). A smoothed dose–response analysis showed that the observed inverse GC risk association of higher plasma vitamin C concentration is more pronounced at higher intake levels of red and processed meats. In the future, the creation of databases concerning the level of dietary consumption and endogenous production of *N*-nitroso compounds and their precursors will allow this observation to be assessed in greater detail.

It is well known that *H.pylori* infection is a major GC risk factor (52–54) and it can interact with vitamin C by reducing its systemic bioavailability (39) and its concentration in gastric juice (40,41). In the present study, *H.pylori* infection status was determined for all cases and controls. Statistical tests for interaction between *H.pylori* positivity and the association of plasma vitamin C with GC risk were not significant, perhaps because of a large percentage of *H.pylori* positive cases (86.5%) and controls (71.2%). Other studies have also observed no statistically significant interactions between Vitamin C (from the diet) and *H.pylori* infection in association with GC risk (55). In another study, *H.pylori* positivity was shown to be a strong risk factor for GC at low levels of dietary vitamin C intake, but not at higher levels (19), implying that any interaction of *H.pylori* status and vitamin C may depend on the level of vitamin C. It may also pertain more to the concentration of vitamin C in gastric juice, since this has been shown to be significantly lower in *H.pylori* positive than *H.pylori* negative subjects, despite similar plasma vitamin C levels in the two groups (40). Given the potential for *H.pylori* infection to modulate the effects of vitamin C (or vice versa), the present study explored the vitamin C–GC risk association stratified by *H.pylori* infection status. Although the results were not statistically significant, they do show divergent GC risk associations based on *H.pylori* status (*H.pylori* positive OR = 0.89; *H.pylori* negative OR = 1.23), probably because of the low number of *H.pylori* negative cases and controls. Together, these results suggest a need to further explore this area, ideally with better powered studies.

By way of an international effort to collect tumor samples and pathology reports the present study can differentiate between GCs on the basis of their anatomical localization and their histological subtype. Both of these factors may play a role in GC etiology (20,21), but have seldom been previously explored. Information from previous case–control studies is mixed with some showing either no effect in the cardia subsite (24) or an inverse association of dietary vitamin C with decreased risk of both anatomical subsites (25–27), while in a prospective setting, a stronger protective effect of dietary vitamin C has been observed in non-cardia GC (30). An inverse association of dietary vitamin C with GC risk has also been observed to be equal in both histological subtypes (25) or to be stronger in the diffuse than in the intestinal (27). Although in the present study no significant associations were observed for plasma vitamin C by either anatomical subtype or histological subsite, the overall direction of effect was negative, particularly in the cardia subsite. This is consistent with findings from a concurrent study based on the entire EPIC cohort showing a non-significant inverse association with the intake of citrus fruits, a rich source of dietary vitamin C, and risk of cardia GCs (5). The observations of the present study may be due to either equal effect or low number of cases.

In the present study, GC risk was associated with plasma vitamin C concentration, but was not related to the level of dietary vitamin C intake. To some degree, this may be because of errors in food composition tables from which dietary vitamin C values were derived or the lack of information on vitamin C intake from dietary supplements. But it could also be due to potential measurement errors in the assessment of dietary vitamin C intake. In order to better account for such errors, a calibration exercise was attempted in the present study. No significant GC risk association was observed, but calibration reduced the OR estimate of dietary vitamin C from 1.09 to 0.95, which is still not comparable with that obtained for plasma vitamin C. This may be because estimation of dietary vitamin C does not account for factors such as efficiency of uptake from the digestive tract or availability from different foods, which may influence overall plasma vitamin C levels. In addition, some cases may have lower plasma vitamin C levels before clinical diagnosis because of potential endogenous consumption of vitamin C by the process of tumor development (8,56) or possible changes in intake patterns of vitamin C-rich foods induced by gastric discomfort from pre-cancerous lesions. Furthermore, plasma vitamin C measures also reflect vitamin C intake derived from dietary supplements. Collectively, these errors may, in part, account for some of the discrepancies in results observed here.

The relationship between dietary and plasma vitamin C is known to be non-linear (57). Within a subgroup of the EPIC study (58), and elsewhere (59), it has been observed that the correlation of dietary and plasma vitamin C is lower at higher levels of vitamin C intake (58). This suggests that the vitamin C–GC risk association may also be non-linear, and that the risk associated with dietary intakes that are lower on the plasma vitamin C curve may be more physiologically relevant. Although this was explored via cubic spline flexible regression models in preliminary analyses for the present study, the results were not different from those presented here. An explanation for this may be found in data from controlled clinical studies showing that plasma vitamin C concentrations start to plateau at levels beyond 80 μmol/l and that dietary intakes >1000 mg/day are associated with complete plasma vitamin C saturation (57)—both of which are higher than the average values of the highest quartiles of plasma and dietary vitamin C observed here. This suggests that in the present study the vitamin C concentrations associated with an inverse GC risk are probably mostly in the linear part of the vitamin C pharmacokinetics curve, before any plateau of plasma values.

A potential limitation of the present study is the relatively short follow-up time. The mean number of years from blood donation to diagnosis was 3.3 years. Cases identified within a short period of time after the start of the study may have been experiencing symptoms leading to dietary changes and hence

alterations in blood vitamin C levels. Here, interaction tests were run in order to assess if data in the first years of follow-up affected the association of plasma vitamin C and cancer risk. However, no statistically significant interactions were observed and elimination of the cases diagnosed in the first 2 years of follow-up did not change the observed negative association, suggesting that the short follow-up time is probably not a major factor in this investigation.

In summary, these results based on the prospective EPIC study are in line with earlier observations that higher plasma vitamin C levels are inversely associated with GC risk. Further studies are also necessary to determine the mechanisms of vitamin C action and any potential interactions with *H.pylori* infection and smoking.

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