Vitamin E concentrations in the human stomach and duodenum – correlation with Helicobacter pylori infection

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Abstract

Background—Vitamin E (α-tocopherol) is an important endogenous antioxidant and may also act as an anticarcinogen. Aim—to determine the vitamin E status of subjects with, and without, gastroduodenal inflammation and Helicobacter pylori infection.

Subjects—36 patients undergoing routine gastroscopy for investigation of dyspepsia.

Methods—High performance liquid chromatography with fluorometric detection was used to determine α-tocopherol values.

Results—In H pylori negative subjects with normal gastroduodenal histology (n=11) median α-tocopherol values (ng/mg tissue weight) were significantly higher in the corpus (16.4, interquartile range (IQR) 8.9-22.6) than in the antrum (3.0, IQR 2.6-6.7, p=0.001) or duodenum (6.7, IQR 2.5-8.4, p=0.001). H pylori infection (n=19) was associated with a reduction in the corpus α-tocopherol values (median 8.3, IQR 4.9-13.7, p<0.05) but there was no significant change in the antral concentrations although this was the main site of inflammation and neutrophil activity. Duodenal α-tocopherol values were not significantly changed in the presence of duodenitis or gastric H pylori infection. α-Tocopherol was not detected in the gastric juice of any of the subjects. Plasma α-tocopherol concentrations in the H pylori negative subjects (median 10.4 mg/l, IQR 7.2-11.9) were not significantly different to the values in the H pylori positive subjects (median 11.1 mg/l, IQR 7.6-12.7).

Conclusions—Concentrations of α-tocopherol in H pylori negative subjects are higher in the corpus than in the antrum or duodenum. In the presence of predominantly antral H pylori infection and neutrophil activity the major change seen is a reduction in corpus α-tocopherol values whilst antral concentrations are maintained. These findings may reflect a mobilisation of antioxidant defences to the sites of maximal inflammation in the stomach.

Keywords: vitamin E, antioxidants, Helicobacter pylori, gastroduodenal inflammation.

It is now recognised that infection with the microaerophilic spiral bacterium Helicobacter pylori plays an important part in human gastroduodenal disease. H pylori is the major cause of chronic gastritis, it is a critical factor in gastric and duodenal ulcer disease, and infection with the organism is also linked to the development of gastric cancer. However, not everyone infected with H pylori develops ulcer disease or gastric cancer. The host factors that are responsible for mucosal protection against this organism are not fully understood.

Antioxidants may play a part in gastric mucosal defence by protecting against damage caused by excessive oxygen derived free radicals (ODFRs), which are highly toxic chemical species. Free radicals can attack DNA and may promote carcinogenesis. Recent studies suggest that ODFRs may have a part to play in the development of H pylori associated gastroduodenal disease, including gastric cancer.

Vitamin E is considered to be the most important antioxidant at the membrane level as it is lipid soluble. It comprises a group of compounds termed tocopherols and tocotrienols, all having a six membered chromanol ring structure and a side chain. α-Tocopherol is the major active form in human systems, accounting for >95% of vitamin E, and is found in all tissues incorporated into cell and organelle membranes. It is transported in the blood by LDL, HDL, and VLDL cholesterol. One of the mechanisms by which ODFRs cause damage is by initiating an autocatalytic chain reaction termed lipid peroxidation, which results in the disruption of biomembranes.

Vitamin E acts as the major chain breaking antioxidant and is able to interfere with the propagation of lipid peroxidation.

In addition to its role as an antioxidant vitamin E may also act as an anticarcinogen through its ability to prevent the formation of N-nitrosoamines, which have been suggested to be important factors in the aetiology of gastric cancer. Furthermore, this vitamin seems to play an immune modulatory part and is capable of increasing natural killer cell activity.

To our knowledge, there is no information to date on vitamin E in normal human gastroduodenal mucosa, or in subjects with H pylori infection. The aim of this study was to measure concentrations of vitamin E in the diet, plasma, gastroduodenal mucosa, and gastric juice of patients with, and without, gastroduodenal inflammation and H pylori infection.

Methods

Patients

Patients undergoing routine gastroscopy for investigation of dyspepsia were recruited to the
study, which was approved by the Northwick Park Hospital ethical committee; written, informed consent was obtained in all cases. Patients were excluded if there was a history of use of acid suppressing treatment or antibiotics within the previous four weeks, if they were taking supplements of vitamins A, C, and E or any other medication known to possess anti-
oxidant activity (for example, allopurinol, captopril, selenium), or if they were taking concomitant therapy with non-steroidal anti-
inflammatory drugs. After gastroscopy, patients were also excluded if there was any evidence of malignancy, oesophageal disease, or special forms of gastritis.

Gastroscopy and sample collection
All gastroscopies were performed by a single operator (PSP) using an Olympus Q20 gastro-
scope. On entering the stomach with the gastro-
scope, a 2–5 ml sample of gastric juice was collected using a sterile trap and placed on ice. Within one hour of collection the gastric juice was centrifuged at 2500 rpm for 10 minutes to remove cell debris, the supernatant was decanted into Eppendorf tubes, and stored at −70°C until analysis.

Three mucosal biopsy specimens were obtained from each gastric compartment: the corpus and the antrum (within 3 cm of the pylorus), as well as the first part of the duodenum. Samples were taken from the distal site first to avoid contamination by blood from the biopsy sites. Two of the specimens from each site were placed in 10% formalin for histo-
logical examination. The one remaining biopsy specimen was immediately snap frozen in liquid nitrogen for vitamin E analysis. Within one hour of collection this sample was weighed and then homogenised in 0·5 ml of ice-cold 0·85% metaphosphoric acid using a mechani-
cal homogeniser; the homogenate was stored at −70°C until analysis.

A fasting blood sample was collected at the time of endoscopy in an EDTA tube for vitamin E assay and placed in ice. This was centrifuged at 2500 rpm for 10 minutes; the plasma was transferred into an Eppendorf tube and stored at −70°C until analysis. A fasting serum sample was also obtained at endoscopy for cholesterol assay.

All chemicals used for the assays were obtained from Sigma Chemical Co (Poole, UK).

Vitamin E (α-tocopherol) assay
Assays were performed within four weeks of sample collection; α-tocopherol is stable when stored at −70°C at least for many months. All samples were assayed in duplicate. For tissue homogenate and gastric juice 0·25 ml aliquots were made up to 1 ml with water and α-tocopherol was extracted in hexane using the method of Buttriss et al. Shearer's method was used for extracting plasma α-tocopherol. Recoveries of α-tocopherol using these extrac-
tion methods are >95%. Retinol acetate in hexane (0·05 μg/ml for tissue homogenate and gastric juice assay, 5 μg/ml for plasma assay) was used as the internal standard. α-Tocopherol was assayed by high performance liquid chromatography (HPLC) using fluorometric detection. The HPLC system consisted of a reverse phase Spherisorb ODS2 5 μm (4·0×100 mm) column (LKB, Milton Keynes, UK) fitted with a Supersac guard cartridge. This was connected to an LKB 2150 HPLC pump with a Rhodoyn 7125 injector valve (Rhodoyn Inc, Cotati, USA). Data were analysed using a Trivector Trio chromatography Computing Integrator (Trivector Systems International Ltd, Sandy, UK). The detector was a Perkin Elmer LS3B Fluorescence spectrometer (Perkin Elmer Ltd, Beaconsfield, UK) with the excitation and emission wavelengths set to 295 nm and 325 nm for α-tocopherol, and 325 nm and 480 nm for retinol acetate, respectively. Using a mobile phase of 95% methanol and 5% sodium acetate (0·1 M, pH 4·25), set at a flow rate of 1 ml/min, α-tocopherol eluted at 7·4 minutes and retinol acetate at 2·8 minutes. Samples were reconstituted in 100 μl of methanol immediately prior to analysis and 50 μl were injected onto the HPLC system. The amount of α-tocopherol in each sample was calculated from standard curves prepared using the ratio of the chromatogram peak area for α-tocopherol to the peak area of the internal standard retinol acetate. The lower limit of detection of α-tocopherol was 18 ng/ml. Reproducibility of the method, expressed as the median (95% confidence intervals) percentage difference between duplicate samples was 9·2 (7·5 to 12·7). The intra-assay coefficient of variation for this assay is 4·4%.16

Cholesterol assay
As α-tocopherol is transported in the blood incorporated in cholesterol, differences in the values between patients may reflect differences in cholesterol values. Therefore, cholesterol corrected values are a more accurate reflection of the amount of α-tocopherol in the blood. Serum cholesterol was assayed by a cholesterol oxidase based method18 using an automated analyser (Kodak Ektam 700XRC). Intra and interassay coefficients of variation were 2·7% and 4%, respectively; the lower limit of detection of cholesterol was 0·1 mmol/l.

Dietary assessment of vitamin E intake
The dietary intake of vitamin E was assessed by means of a computer software program, DIETQ (Trimuviel Software, Warrington, UK). This is based on a food frequency question-
naire developed by the MRC Epidemiology group in Cardiff, Wales. On entry into the study, all patients were asked to complete a detailed questionnaire regarding their average food intake over a seven day period. Subsequent analysis of the nutrient composi-
tion of this diet is based on a database from McCance and Widdowson's The composition of foods.
TABLE I Demographic characteristics of patients

<table>
<thead>
<tr>
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<th>H pylori −ve</th>
<th>H pylori +ve</th>
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<tbody>
<tr>
<td>Number</td>
<td>Normal controls (11)</td>
<td>Gastritis only (10)</td>
</tr>
<tr>
<td>Mean age (y, range)</td>
<td>57 (34-81)</td>
<td>45 (25-68)</td>
</tr>
<tr>
<td>Males (%)</td>
<td>4 (36)</td>
<td>8 (69)</td>
</tr>
<tr>
<td>Smokers (%)</td>
<td>1 (9)</td>
<td>2 (22)</td>
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Histology
The gastroduodenal biopsy specimens were examined by an experienced pathologist (ABP) with a special interest in upper gastrointestinal pathology who was unaware of the endoscopic findings. Biopsy tissue, fixed in 10% formalin and embedded in paraffin wax, was sectioned at 3 μm and stained with haematoxylin and eosin, as well as a cresyl-fast violet stain for H pylori. The gastritis was graded according to the Sydney system.21 This grades the severity of inflammation, activity (the degree of polymorph neutrophil infiltration), atrophy, and intestinal metaplasia on a scale of 0 to 3. A subsequent ‘gastritis score’ for each biopsy site was obtained by combining the scores for the four individual characteristics (maximum possible score=12). In accordance with the Sydney system, the density of H pylori infection was also graded semi-quantitatively on a scale of 0 to 3.

Duodenitis is almost invariably present in duodenal ulceration and is believed to be part of the same pathophysiological spectrum.22 The degree of duodenal inflammation was graded according to Whitehead.23

Statistics
The Shapiro-Wilks test was used to assess the normality of the distribution of the results. The non-parametric Mann-Whitney U test was used to compare vitamin E values between groups. The Wilcoxon paired signed rank test was used for comparing vitamin E values and gastritis scores within groups. Two tailed statistics were used throughout and a p value of less than 0.05 was considered to be significant.

Results
Forty patients were investigated, of whom four were excluded after the results of histology (one – gastric cancer, two – reactive gastritis, one – lymphocytic gastritis). Of the remaining 36 patients, 11 had normal gastroduodenal histology with no evidence of H pylori infection. Fourteen patients had chronic gastritis on histology but a normal duodenum; 10 of these patients (71%) were H pylori positive. Ten patients had duodenal ulceration or histological evidence of duodenitis, or both, of whom nine (90%) were H pylori positive. One patient had H pylori positive gastric ulceration. The H pylori negative patients with chronic gastritis or duodenitis were excluded from further analysis, as was the one patient with gastric ulceration, as the small numbers in each group did not allow for a valid evaluation of their results.

The results presented, therefore, are for 11 H pylori negative patients with normal gastroduodenal histology (gastritis score=0, duodenitis score=0) and 19 H pylori positive patients, all of whom had an associated chronic gastritis. Of the second group, 10 had chronic gastritis only (duodenitis score=0) and nine patients had chronic gastritis plus duodenitis (median duodenitis score=1, range 1–3). Table I shows the demographic characteristics of these patients.

Vitamin E
The median daily dietary intake of vitamin E in the normal H pylori negative patients was 4.2 mg (interquartile range IQR 3.4–6.1), which was not significantly different from the intake in chronic gastritis only patients (3.8 mg, IQR 3.2–5.8), chronic gastritis plus duodenitis patients (5.7 mg, IQR 3.7–7.4) or all H pylori positive patients (4.5 mg, IQR 3.3–6.1).

Table II shows the results for plasma α-tocopherol values. No significant differences were seen in the plasma concentrations of α-tocopherol or the α-tocopherol/cholesterol ratios between patients with or without H pylori infection.

Gastric mucosal results (Table II) showed that in H pylori negative patients with normal gastroduodenal histology the α-tocopherol values in the corpus were significantly higher than in the antrum (p=0.001). H pylori infection (n=19) was associated with a reduction in the corpus concentrations of α-tocopherol (median 8.3, IQR 4.9–13.7), which were significantly lower compared with the normal group (p<0.05). This reduction in corpus α-tocopherol values (Table II) was seen both in subjects with chronic gastritis only (p<0.05) and in those with chronic gastritis plus duodenitis (p=0.03). α-Tocopherol values in the antrum did not change significantly in the presence of H pylori infection. Further analysis of the H pylori positive patients showed no significant differences in the gastric α-tocopherol values between patients with chronic gastritis only and those with chronic gastritis plus duodenitis.

Duodenal α-tocopherol values in histologically normal, H pylori negative, patients were significantly lower than corpus values (p=0.001) but were similar to antral values (see Table II). The amount of α-tocopherol in the duodenum did not change significantly in the presence of gastric H pylori infection or

![Table I](https://example.com/table1.png)

![Table II](https://example.com/table2.png)
duodenal inflammation. There were no detectable levels of α-tocopherol in the gastric juice of any of the patients.

No significant differences were seen between smokers and non-smokers, or men and women in the plasma, tissue or gastric juice α-tocopherol values.

Histology

The histological results showed that the H. pylori positive patients had an antral predominant gastritis, with the gastritis score being higher in the antrum (median 3, IQR 3–5) than in the corpus (median 2, IQR 1–2; p<0.0002). The scores for neutrophil activity and density of H. pylori infection were also significantly higher in the antrum. Median activity score in the antrum was 2 (IQR 1–2) compared with a score of 0 (IQR 0–1) in the corpus (p<0.002). The median scores for density of H. pylori infection were 2 (IQR 1–2) in the antrum and 1 (IQR 0–2) in the corpus (p<0.003).

Discussion

That vitamin E may play a part in gastroduodenal disease was suggested by studies performed in rats showing a protective effect for α-tocopherol against gastric mucosal injury induced by a variety of agents.24 25 Further support comes from human studies carried out in the pre-H. pylori era, which have shown that low concentrations of vitamin E in the plasma are associated with premalignant lesions33 such as chronic atrophic gastritis27 and gastric dysplasia.28 Longitudinal studies have further shown that low plasma concentrations of vitamin E are associated with an increased risk of developing gastric cancer.29 30 There are few data available on the dietary intake of vitamin E in gastroduodenal disease but one Canadian study did not find any relation with the risk of gastric cancer.31

We are aware of only one previous study, published in abstract form, in which vitamin E concentrations were measured in human gastroduodenal mucosa.32 Ten patients were investigated (three with gastric ulcers and seven with duodenal ulcers) with mucosal tissue being obtained from the ulcer margins and also from a ‘corresponding normal area’. Concentrations of α-tocopherol were found to be higher at the ulcer margins although no TBPpm difference was seen in values of α-tocopherol quinone, the oxidised product. It is difficult to interpret these results as endoscopically ‘normal’ tissue from the stomach of patients with peptic ulceration is unlikely to be histologically normal and, furthermore, there is no indication given of the severity of inflammation or presence of H. pylori at the sites where vitamin E values were measured.

The results presented in this paper show that in H. pylori negative subjects with histologically normal gastroduodenal mucosa the values of α-tocopherol in the corpus are significantly higher than in the antrum or duodenum. This ‘α-tocopherol gradient’ is a similar finding to that reported in the gastroduodenal mucosa of the rat, with α-tocopherol concentrations being highest in the fundus, lower in the antrum, and lowest in the duodenum.33

In the presence of H. pylori infection, despite no differences in the dietary intake of vitamin E, the values of α-tocopherol in the gastric corpus mucosa are reduced; antral values of α-tocopherol did not change significantly. No changes were seen in the duodenal concentrations of α-tocopherol in the presence of H. pylori infection.

Why the major change in α-tocopherol concentrations occurred in the corpus in the presence of antral predominant H. pylori infection, inflammation and neutrophil activity is not clear. The antrum would be expected to be the major site of increased free radical activity as production of ODFRs in human gastroduodenal disease seems to arise largely from neutrophils that have been activated by H. pylori.6 7 There is a complex process of redox cycling of the various antioxidants and it seems that the antioxidant status of vitamin E is maintained at the expense of other antioxidants.34 This may explain why we did not observe any changes in the plasma α-tocopherol concentrations in our H. pylori positive patients. It is possible that a similar process occurs in the gastroduodenal mucosa to maintain α-tocopherol values. However, this would not accurately explain the observed phenomenon of corpus values falling in the face of inflammation occurring at a distant site in the stomach. One explanation might be that the changes observed reflect a mobilisation of antioxidant defences to the sites of maximal free radical activity – that is, from the corpus to the antrum and duodenum – possibly involving the gastric microcirculation. A similar phenomenon has been observed in lung studies with rats in whom pulmonary vitamin E values increased in the face of oxidant stress.35 Chow et al showed that rats exposed to tobacco smoke had significantly higher concentrations of vitamin E in the lungs than controls, and also that the duration of exposure to the tobacco smoke correlated with increases in pulmonary vitamin E values.

An alternative explanation for our findings may lie with the plasma membrane α-tocopherol binding protein (TBP_{pm}) which, it is hypothesised, is involved in the uptake of α-tocopherol by cell membranes.36 Although there are no data available on TBP_{pm} in human gastroduodenal tissue, it is conceivable that there may be changes in the regulation of this protein in the presence of disease, which will affect the values of α-tocopherol in the tissues.

We were unable to detect α-tocopherol in the gastric juice of H. pylori negative or positive subjects. This is in contrast with the findings reported with another antioxidant, vitamin C.37 The reason for this may be the different solubility of these two antioxidants in the mainly aqueous gastric juice; vitamin C is highly water-soluble and vitamin E is not.

Subjects with chronic gastritis but without H. pylori infection constitute a small but interesting group of patients. Unfortunately,
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we do not have any vitamin E data for these patients. In the absence of such information it is difficult to ascertain whether the changes that we have demonstrated in gastric α-tocopherol concentrations are specific to H pylori infection compared with gastritis in general. The results of further studies may clarify the situation.

In summary, corpus values of vitamin E are higher than antral or duodenal concentrations in H pylori negative subjects with normal gastroduodenal histology. This may be of importance as infection with H pylori affects the corpus less often than the antrum and also induces a less severe inflammatory response in the corpus. 38 In the presence of predominantly antral H pylori infection the only change seen is a reduction in the corpus α-tocopherol values, with antral values being maintained. These findings may reflect a mobilisation of antioxidant defences to the sites of maximal inflammation and neutrophil activity.

The preliminary results from this study were presented at the British Society of Gastroenterology Spring meeting in 1993 and published in abstract form in Gut 1993; 34 (suppl 1): S34. PSP is partly funded by a grant from Glaxo Research and Development, UK.


