Effect of Proton Pump Inhibitors on Vitamins and Iron

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Vitamin C is actively secreted in human gastric juice. Proton pump inhibitor therapy lowers the concentration of vitamin C in gastric juice and the proportion of the vitamin in its active antioxidant form i.e., ascorbic acid. This has secondary effects on intragastric nitrite chemistry, resulting in a rise in gastric juice nitrite levels. There is also some evidence that proton pump inhibitors may reduce the bioavailability of ingested vitamin C. The effect of proton pump inhibitors on vitamin C and nitrite chemistry is more marked in Helicobacter pylori-infected subjects. Proton pump inhibitors also reduce the absorption of vitamin B₁₂, probably by inhibiting intragastric proteolysis and, thus, its release from food required prior to binding to R-proteins and gastric intrinsic factor. Under certain circumstances, the treatment may lower serum vitamin B₁₂ levels. Proton pump inhibitor therapy reduces the absorption of non-heme iron and this effect has been employed in the management of hemochromatosis. It may also retard clinical response to iron supplementation.

INTRODUCTION
Proton pump inhibitors are powerful inhibitors of gastric acid secretion, raising intragastric pH by several units and reducing the intragastric hydrogen ion concentration by several hundred or thousand fold. As a consequence, they have the potential to modify essential micronutrients as they pass through the stomach and alter their activity within the gastric lumen as well as their bioavailability. In this chapter, the effect of proton pump inhibitor therapy on vitamin C, vitamin B₁₂, and iron is reviewed.

PROTON PUMP INHIBITORS AND VITAMIN C
Humans are unable to synthesize vitamin C and rely on obtaining the essential vitamin from their dietary intake. Proton pump inhibitors affect vitamin C and its functions within the stomach and as a consequence, may also influence its bioavailability. Prior to discussing the effects of proton pump inhibitor therapy, it is necessary to provide some background on the presence and functions of vitamin C in human gastric juice.

GASTRIC JUICE VITAMIN C AND ITS FUNCTIONS
Vitamin C is actively secreted by the gastric mucosa and in the healthy stomach, its concentration in gastric juice is higher than that in plasma (1,2). In addition, most of the vitamin C present in gastric juice is in its biologically active antioxidant form, ascorbic acid, and only a small proportion in the oxidized form, dehydroascorbic acid (1,2). Vitamin C is thought to be secreted mainly by the mucosa of the distal, antreal region of the stomach, though the exact epithelial cells responsible for the secretory process are unclear (3). The ascorbic acid is assumed to act as a general antioxidant in gastric juice; however, it has other specific functions related to both nitrite chemistry and iron chemistry either of which may represent its primary evolutionary benefit.

Effect on gastric juice nitrite
The ascorbic acid actively secreted by the gastric mucosa influences the concentrations of nitrite in the juice. The main source of nitrite entering the stomach is swallowed saliva. The latter has high concentrations of nitrite arising from the entero-sali-vary recirculation of dietary nitrate and its reduction to nitrite by bacteria colonizing the dorsal surface of the tongue (4–10). Dietary nitrate is absorbed from the small intestine and mixes with nitrate derived from the breakdown of endogenously produced nitric oxide. Approximately, 25% of all the circulating nitrate is taken up by the salivary glands and secreted into the mouth (4). Twenty percent of this nitrate present in saliva is reduced to nitrite by the buccal bacteria (4).

When saliva is swallowed and encounters the acidic pH of the stomach, it is converted to nitrous acid and nitrosating species (11,12). The latter are able to react with a variety of nitrosatable compounds to produce potentially carcinogenic N-nitroso compounds (11,12). However, ascorbic acid effectively competes for these nitrosative species, converting them to nitric oxide and, in the process, is oxidized to dehydroascorbic acid (13–17).
In the healthy acid-secreting stomach, very little nitrite can be detected in gastric juice despite large amounts being delivered in swallowed saliva (18). This is due to the ascorbic acid in the juice rapidly converting the nitrite to nitric oxide and the latter being absorbed by the gastric mucosa (19). This ability of gastric juice ascorbic acid to prevent acid-catalyzed formation of potentially mutagenic N-nitroso compounds is considered to be important in protecting against gastric carcinogenesis. The only place in the healthy acid-secreting stomach in which the concentration of nitrite exceeds that of ascorbic acid is at the cardia in which the saliva first enters the stomach (20). It has been suggested that this luminal chemistry might contribute to the high incidence of mutagenesis and neoplasia at this anatomical site in the healthy acid-secreting stomach (20).

The ability of ascorbic acid to remove nitrite from gastric juice by converting it to nitric oxide is highly pH dependent (19). This is due to the fact that the nitrosating species, with which ascorbic acid reacts, only exist at pH lower than the pKa for nitrous acid which is 3.5 (4). Consequently, in gastric juice of pH>4, the nitrite entering the stomach in swallowed saliva remains as nitrite and causes an increase in its gastric juice concentration (18). The original Correa hypothesis of gastric cancer developing in patients with atrophic gastritis hypothesized a central role for the elevated gastric nitrite concentration (21). It proposed that nitrosating bacteria colonizing the achlorhydric stomach converted the nitrite to carcinogenic N-nitroso compounds (22). This bacterial mechanism of generation of N-nitroso compounds occurring at neutral pH should be distinguished from acid-catalyzed chemical nitrosation, already discussed above.

**Effect of gastric juice vitamin C on iron absorption**

More than 25 years ago, a number of investigators observed that ascorbic acid increased the absorption of non-heme iron (23). This promotion of iron absorption was seen when ascorbic acid was coadministered with pure elemental iron or with an iron containing meal. Later studies indicated that vitamin C chelates non-heme iron in the stomach and, thereby, maintains it in solution when it encounters the higher pH of the duodenum and thus is able to be absorbed at that site (24). These observations were made before it was recognized that ascorbic acid was actively secreted in gastric juice. It seems likely that the ascorbic acid secreted in gastric juice will promote non-heme iron absorption similar to ingested ascorbic acid, though this has not been directly studied to date.

**Effect of gastric disorders on gastric juice vitamin C**

*Helicobacter pylori* infection of the gastric mucosa, which affects majority of the world's population, lowers the gastric juice concentration of vitamin C and reduces the proportion which is in the biologically active antioxidant form, ascorbic acid (1,2). Eradication of *H. pylori* infection reverses most of these abnormalities (2,25,26). The effect of *H. pylori* infection on gastric juice vitamin C is most marked in subjects with atrophic gastritis (27,28). A number of factors may contribute to a lower gastric juice vitamin C in patients with *H. pylori* infection and atrophic gastritis (27,28). This includes inflammation of the gastric mucosa which will tend to oxidize ascorbic acid to dehydroascorbic acid. Ascorbic acid is unstable at high pH and thus more susceptible to degradation as patients with *H. pylori*-induced atrophic gastritis and hypochlorhydria (29). Even in patients without atrophic gastritis, the high concentrations of ammonia produced by the bacterium may increase the pH close to the epithelium increasing the vitamin's degradation locally.

There is some evidence that the altered metabolism of vitamin C in patients with *H. pylori* infection and atrophic gastritis may affect circulating vitamin C concentrations. In a large epidemiological study, the serum concentration of vitamin C relative to the dietary intake of vitamin C was substantially lower in *H. pylori*-infected vs. -uninfected subjects and this effect was most marked in older patients with *H. pylori* infection with a higher prevalence of atrophic gastritis (30).

**EFFECT OF PROTON PUMP INHIBITORS ON GASTRIC JUICE VITAMIN C**

We have investigated the effects of 40 mg omeprazole on gastric juice concentrations of vitamin C in healthy volunteers following a 4-week course of the medication (18). The analysis was performed 2h after the last dose of omeprazole. The omeprazole produced a profound rise in intragastric pH in all subjects. The median intragastric pH increased from 1.4 pre-omeprazole to 7.2 (range 3.5–8.5) on omeprazole (*P*<0.001). The omeprazole also lowered the concentration of vitamin C in fasting gastric juice from 5 µmol/l (1.2–21) pre-omeprazole to 3 µmol/l (0.8–11.3) on omeprazole (*P*<0.005). The omeprazole had more profound effect on the concentration of ascorbic acid, lowering it from 3.8 to 0.7 µmol/l. The omeprazole, thus, markedly reduced the proportion of vitamin C in its biologically active antioxidant form of ascorbic acid. These changes in the pH and ascorbic acid concentration of the fasting gastric juice also influenced the nitrite concentration in gastric juice, raising it from 0 (0–12) to 13 µg/ml (0–50) (*P*<0.001). In this particular study, 9 of the subjects had evidence of *H. pylori* infection and 11 were *H. pylori* negative. Analysis of these two groups indicated that the fall in fasting gastric juice total vitamin C was only significant in those with *H. pylori* infection. However, in both groups, there was a significant reduction in the proportion of the total vitamin C in its biologically active absorption form of ascorbic acid.

We undertook a further study to investigate, in more detail, the influence of *H. pylori* infection on the changes induced by omeprazole therapy on gastric juice vitamin C and related chemistry (31). In this study, we examined subjects 24h after the last dose of a 4-week course of omeprazole 40mg/day. Pre-omeprazole, the *H. pylori*-positive and -negative subjects were similar for all parameters. During omeprazole treatment, *H. pylori*-positive subjects recorded a higher intragastric pH than *H. pylori*-negative subjects (7.8 vs. 3.0) (*P*<0.0001).
During omeprazole treatment, the fasting gastric juice total vitamin C level in the *H. pylori*-positive subjects fell from 3.7 (1.7–6.2) pre-omeprazole to 1.8 μg/ml (0.7–3.4) (*P* < 0.009) but was unchanged in *H. pylori*-negative subjects, at 3.4 (1.3–14.8) vs. 4.2 μg/ml (1.8–19.5) pre-omeprazole. The fasting gastric ascorbic acid level also fell in the *H. pylori*-positive group from 2.0 (1.3–5.1) pre-omeprazole to 1.2 μg/ml (0–21.4) on omeprazole such that the ascorbic acid became undetectable in 5 of the 10 subjects. In *H. pylori*-negative subjects, the fall was less marked and was not statistically significant.

The *H. pylori*-positive vs. -negative subject also differed with respect to the changes in intragastric nitrite on omeprazole. The *H. pylori*-positive subjects showed a greater rise in intragastric nitrite than the *H. pylori*-negative subjects following the administration of a physiological dose of nitrate. A positive correlation was also observed between the degree of elevation of intragastric pH on omeprazole and the degree of elevation in intragastric nitrite following the nitrate administration when all subjects were analyzed together.

These studies indicate that omeprazole causes a fall in the concentration of total vitamin C in gastric juice and in the proportion of that vitamin C in its antioxidant form. In addition, the effects are much more marked and possibly confined to subjects with *H. pylori* infection. What is the mechanism of the fall in gastric juice total vitamin C and in ascorbic acid during proton pump inhibitor therapy? Vitamin C is relatively unstable at neutral pH (32,33). This is because its induced form, dehydroascorbic acid, can be readily hydrolyzed to 2,3-diketogulonic acid at pH >4, a reaction which is catalyzed by trace metals such as iron or copper, which are present in gastric juice (32–34). Though dehydroascorbic acid can be reduced back to ascorbic acid, this is not possible with 2,3-diketogulonic acid which causes a loss in total vitamin C. Several mechanisms may explain why the effect of omeprazole on gastric juice vitamin C and ascorbic acid was more marked in *H. pylori*-infected vs. -uninfected subjects. First, omeprazole causes a more marked rise in intragastric pH in the presence of *H. pylori* and this will contribute to the instability and degradation of the vitamin C (35). Furthermore, it is known that omeprazole therapy allows extension of the gastritis into the body mucosa and this more extensive inflammation may contribute to the destruction of vitamin C (36).

Are these changes in gastric juice vitamin C of clinical significance? It is proposed that vitamin C may protect against the conversion of nitrite to N-nitroso compounds by bacteria which may colonize the achlorhydric stomach and there is some evidence for this from both *in vitro* and *in vivo* studies (37–39).

We, and others, have shown that the bacterial count rises in the gastric juice of patients on omeprazole and again this rises more markedly in subjects with *H. pylori* infection (39). It is, therefore, possible that the loss of vitamin C may predispose to bacterial generation of N-nitroso compounds which could contribute to the development of gastric neoplasia. All of the changes induced by omeprazole were most marked in the presence of *H. pylori* infection; the rise in pH, the rise in nitrite, the fall in vitamin C, and the rise in bacterial count (31). Consequently, it would appear that the risk of bacterial N-nitrosation is much more likely in subjects treated with omeprazole who have *H. pylori* infection. These findings would support a policy of eradicating *H. pylori* infection prior to long-term proton pump inhibitor therapy. The effects of changes in intragastric vitamin C on iron absorption will be discussed below.

**Effect of proton pump inhibitor therapy on circulating levels of vitamin C**

We have also observed that omeprazole may produce a slight reduction in the serum concentration of vitamin C (18,40). This effect again tended to be more evident in *H. pylori*-infected vs. -uninfected subjects. The fall in serum vitamin C on omeprazole therapy may be explained by reduced bioavailability of ingested vitamin C. Vitamin C is not synthesized in humans and the essential vitamin has to be obtained from the diet. Ingested vitamin C will be subjected to the same intragastric environment as that secreted by the gastric juice and, therefore, liable to be metabolized to 2,3-diketogulonic acid which cannot be converted back to bio-active vitamin C (3,41). Again, this effect would be expected to be more marked in those with *H. pylori* infection for whom the rise in intragastric pH is more marked. In addition, the extension of the gastritis into the body of the stomach in the *H. pylori*-infected subjects on omeprazole may increase the metabolism of vitamin C and contribute to systemic depletion. Again, these observations would support eradication of *H. pylori* infection prior to a long-term proton pump inhibitor therapy, particularly in elderly or frail subjects who have an increased propensity to vitamin C deficiency.

**Summary**

Proton pump inhibitor therapy lowers intragastric concentrations of vitamin C, particularly in the biological active antioxidant form of ascorbic acid. This is mainly because of the relative instability of the vitamin at high pH. As a consequence of the lowered ascorbic acid concentration and high pH, there is an accumulation of salivary nitrite also within the lumen of the stomach. There is some evidence that proton pump inhibitor therapy also lowers circulating concentrations of vitamin C which may be because of the intragastric degradation reducing bioavailability of dietary vitamin C. The clinical significance of the effect of proton pump inhibitors on intragastric and systemic vitamin C is unclear, but any adverse effects are likely to be most marked or limited to those with a coexistent *H. pylori* infection, which accentuates all the effects of proton pump inhibitor therapy on vitamin C metabolism.

**PROTON PUMP INHIBITOR THERAPY AND VITAMIN B₁₂**

Vitamin B₁₂ is present in foodstuffs bound to proteins. The vitamin requires to be released from these proteins and then bound...
to R-proteins and intrinsic factor in order to be absorbed in the terminal ileum (42) Gastric acid facilitates the proteolytic process involved in releasing the vitamin from the proteins in ingested food. There are, therefore, theoretical reasons why the inhibition of gastric acid secretion by proton pump inhibitor therapy and increase in intragastric pH would reduce the bioavailability of dietary Vitamin B12.

A number of studies have investigated the potential effect of proton pump inhibitor therapy on vitamin B12 absorption. Healthy subjects treated with omeprazole 20 or 40 mg daily for 2 weeks showed decreased vitamin B12 absorption as measured by the modified Schilling test (43). The absorption of the vitamin was reduced from 3.2 to 0.9% with 20 mg omeprazole and from 3.4 to 0.4% with 40 mg omeprazole. Longer-term studies have shown that vitamin B12 concentrations remain within the normal limit within the initial 3 years of treatment (44,45), but longer duration of therapy has shown a small but significant downward trend (46). Another study observed no decrease in serum vitamin B12 in 25 patients receiving omeprazole 20–60 mg per day for 18–56 months (47) Serum vitamin B12 levels have been monitored for long-term acid-suppressive therapy for the Zollinger–Ellison syndrome (41). The mean follow-up was 4.5 years in 111 patients taking omeprazole and 10 years in 20 patients receiving H2-receptor antagonists. Eight patients developed subnormal serum vitamin B12 levels during follow-up and the vitamin B12 levels correlated with a degree of acid suppression.

Sagar et al. (48) investigated the association between CYP2C19 polymorphism and serum levels of vitamin B12 in patients during long-term omeprazole treatment. Mutations of this gene inhibit the metabolism of omeprazole, increasing its serum concentrations and its acid-suppressive effects. In the 68 patients on long-term (>1 year) therapy with 20 mg omeprazole daily, serum vitamin B12 levels were lower in those heterozygous for the mutation (n = 19) compared with those with no mutation (n = 49) (246±71 vs. 305±98 pmol/l), (P = 0.01). In one patient who was homozygous for the mutation, vitamin B12 levels fell during the first 15 months of treatment from 360 to 178 µmol/l.

Shenk et al. (49) monitored the vitamin B12 level in 49 H. pylori-positive GERD patients who were treated with omeprazole for a mean of 61 months. Fifteen patients developed gastric atrophy and in these, the serum vitamin B12 fell significantly from 340 to 285 µmol/l. In the 34 patients who did not develop atrophy, the serum vitamin B12 levels remained constant, being 312 µmol/l pre-omeprazole and 341 µmol/l after omeprazole.

The above studies indicate that under certain circumstances, proton pump inhibitor therapy may cause a fall in the circulating vitamin B12 level. Such circumstances include patients who are genetically slow metabolizers of omeprazole, those with H. pylori gastritis at risk of developing atrophic gastritis, and those on high doses of omeprazole for several years. At present, there is little evidence to suggest vitamin B12 monitoring is required routinely in all patients on proton pump inhibitor therapy though the effects of long-term treatment should be kept under review.

PROTON PUMP INHIBITORS AND IRON ABSORPTION

Iron is absorbed from the diet either in the form of heme or non-heme iron and gastric hydrochloric acid plays a role in the processes involved in the absorption of the latter. Gastric acid facilitates the dissociation of iron salts from food. Gastric acid also reduces ferric iron to the more soluble ferrous form and allows formation of complexes with ascorbate, sugars, and amines that further facilitate its absorption in the duodenum. Evidence for a role of acid in iron absorption comes from studies in which the addition of acid has improved non-heme iron absorption in patients with achlorhydria (50). In vitro studies have shown that the release of radio-labeled iron from bread increased linearly with increase in acidity with pH < 2.5 but little iron was released at higher pH (51). In linked in vivo studies, a close correlation was observed between iron absorption and the capacity of gastric juice to release food iron (51). Inhibition of acid secretion by cimetidine resulted in a significant reduction of absorption of non-heme iron from a test meal in controls and in patients with hepatic hemochromatosis (52). Studies in laboratory rats showed that omeprazole inhibited the absorption of ferrous iron and food iron in rats on an iron deficient diet but not in those on a normal diet (53). The absorption of ferric iron was not affected.

Despite the above theoretical considerations, there is relatively little data to indicate that proton pump inhibitor therapy causes iron deficiency. One case report included two patients who failed to respond to oral iron treatment by taking proton pump inhibitor, but responded when the proton pump inhibitors were withdrawn (54).

A recent study was conducted in patients on the effects of proton pump inhibitors on iron absorption in patients with hemochromatosis. The proton pump inhibitor reduced the absorption of non-heme iron from a test meal by approximately 50% (55). The proton pump inhibitor therapy also was significantly reduced with the volume of blood requiring to be venesected annually from 2.51 before proton pump inhibitor therapy to 0.51 during proton pump inhibitor therapy. Despite these reports, there is no report that proton pump inhibitor therapy under normal clinical circumstances results in the occurrence of iron deficiency. However, in patients with iron deficiency demanding increased iron absorption proton pump inhibitor therapy may retard replenishment of the iron pool.

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patients with and without precancerous conditions of the stomach. 


