# Proton Support ™

Vitamin and mineral deficiencies and intestinal flora imbalance caused by chronic use of proton pump inhibitors

Scientific overview

Companion Therapeutics

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## 1. DESCRIPTION AND USE

PROTON Support is a dietary supplement specifically designed to help maintain the balance of vitamins, minerals and intestinal flora, typically affected by the prolonged use of proton pump inhibitors (PPIs).

## 2. PHARMACOLOGICAL BACKGROUND

Proton pump inhibitors (PPIs) are one of the most widely used classes of drugs. PPIs have proven to have a very favorable safety profile and it is unusual for a patient to stop these drugs because of side effects. However, increasing numbers of patients are chronically taking PPIs for one year or more for the treatment of gastroesophageal reflux disease and a number of other common persistent conditions, therefore the long-term potential adverse effects are receiving increasing attention. (Ito & Jensen, 2010)

### 2.1. Calcium

Epidemiological studies have shown an important relationship between chronic use of Proton Pump Inhibitors (PPIs) and increased risk of fractures. In May 2010, the Food and Drug Administration (FDA) released the following safety announcement: *"Possible Increased Risk of Bone Fractures with Certain Antacid Drugs"*. (FDA Consumer Health Information, 2010) In this publication, FDA warns: *"There is a possible increased risk of fractures of the hip, wrist, and spine if you take certain drugs for heartburn, acid reflux, or ulcers. The drugs belong to a class of medications called proton pump inhibitors (PPIs), which work by reducing the amount of acid in the stomach."*.

| Prescription PPIs | | | |
|---|---|---|---|
| Nexium | Prevacid | Dexilant | Protonix |
| Prilosec | Aciphex | Zegerid | Vimovo |

| Over-the-counter PPIs | | |
|---|---|---|
| Prilosec OTC | Zegerid OTC | Prevacid 24H |

*Table 1. List of branded PPIs in the US (Prescription and OTC)*

FDA warning is based on seven published studies, six of which reported an increased risk of fractures of the hip, wrist, and spine with the use of PPIs. Two of the earliest and largest studies, investigating this potential correlation, were published in 2006. In the first study, examining a population of 124,655 fractures of any type and 373,962 controls matched on age and gender, the authors concluded that the use of PPIs was associated with an increase in fracture risk for use within the last year (OR = 1.18) for overall fracture risk; (OR = 1.45) for hip fractures; and (OR = 1.60) for spine fractures. (Vestergaard, Rejnmark, & L., 2006) In the second study, examining a population of over

13,000 hip fracture cases and over 135,000 controls over the age of 50, the authors concluded that long-term (over 1 year) PPIs use was associated with an increase in hip fractures (AOR = 1.44). (Yang, Lewis, Epstein, & Metz, 2006)

Other studies cited by FDA, as support for releasing the safety announcement include: (Targownik, et al., 2008) (Corley, Kubo, Zhao, & Quesenberry, 2010) (Gray, et al., 2010) (Yu, et al., 2008). Their results are summarized in the table 2. (Johnson, 2014)

| Study | Fracture | Odds ratio [1] | Duration of PPI Tx |
|---|---|---|---|
| (Vestergaard, Rejnmark, & L., 2006) | All | 1,18 | < 1 year since last use |
| | Hip | 1,45 | |
| | Spine | 1,6 | |
| (Yang, Lewis, Epstein, & Metz, 2006) | Hip | 1,44 | > 1 year |
| | Hip | 2,65 | > 1 year with high dose |
| | Hip | 1,22 | 1 year |
| | Hip | 1,59 | 4 years |
| (Targownik, et al., 2008) | All | 1,92 | ≥ 7 years |
| | Hip | 1,62 | 5+ years |
| | Hip | 4,55 | 7+ years |
| (Corley, Kubo, Zhao, & Quesenberry, 2010) | Hip | 1,3 | > 2 years |
| | Hip | 1,41 | > 2 years with high dose |
| (Gray, et al., 2010) | All | aHR=1,25 | Mean 7,8 years |
| | Hip | aHR=1,00 | |
| | Spine | aHR=1,47 | |
| | Wrist | aHR=1,26 | |
| (Yu, et al., 2008) | Hip (F) | aHR=1,16 | Female mean: 7,6 years |
| | Hip (M) | aHR=0,62 | Male mean: 5,6 years |
| | Nonspine (F) | aHR=1,34 | |
| | Nonspine (M) | aHR=1,21 | |

*Table 2. Summary results of epidemiologic studies cited by FDA as support for releasing the safety announcement.*

As a result of FDA announcement, the labels for both the prescription and the over-the-counter PPIs were revised to include new safety information:

[1] An odds ratio (OR) is a measure of association between an exposure and an outcome. The OR represents the odds that an outcome will occur given a particular exposure, compared to the odds of the outcome occurring in the absence of that exposure.

*"Bone Fracture: Long-term and multiple daily dose PPI therapy may be associated with an increased risk for osteoporosis-related fractures of the hip, wrist or spine."* (AstraZeneca Pharmaceuticals LP, 2012)

*"Several published observational studies suggest that proton pump inhibitor (PPI) therapy may be associated with an increased risk for osteoporosis-related fractures of the hip, wrist, or spine. The risk of fracture was increased in patients who received high-dose, defined as multiple daily doses, and long-term PPI therapy (a year or longer)."* (AstraZeneca Pharmaceuticals LP, 2012)

*"Patients should use the lowest dose and shortest duration of PPI therapy appropriate to the condition being treated. Patients at risk for osteoporosis-related fractures should be managed according to established treatment guidelines."* (AstraZeneca Pharmaceuticals LP, 2012)

An additional meta-analysis, published one year after the FDA announcement, also supports the initial hypothesis that a correlation between PPI intake and fracture risk (hip, spine, and any-site fractures) exists. (Yu, Bauer, Bain, & Bauer, 2011) The meta-analysis considered 11 studies and identified an overall odds ratio of 1.30 for all fracture types combined. (Yu, Bauer, Bain, & Bauer, 2011)

Other prospective study published in 2012, also showed the association between chronic use of PPIs and an increased risk of hip fracture, particularly among women with a history of smoking. (Khalili, et al., 2012)

More recently, in 2016, a new meta-analysis was published in Osteoporosis international under the title: *"Proton-pump inhibitors and risk of fractures: an update meta-analysis"*. (Zhou, Huang, Li, Sun, & Liu, 2016) This time the revision included eighteen studies involving a total of 244,109 fracture cases. Again, the results of this study showed that PPIs use was associated with a moderately increased risk of hip, spine, and any-site fracture. Rather than just reporting a similar finding as the previous meta-analysis, with the benefit of additional data, this meta-analysis would provide high statistical power to confirm this moderate, but consistently observed association with fracture risk.

Although the likelihood of sustaining a fracture following PPIs intake may seem fairly low, the implications for public health are substantial. This has multiple reasons: PPIs represent the third most commonly prescribed medication in the United States with more than 7 billion doses sold in 2015, according to IMS. PPIs are also available as over-the-counter formulations. Furthermore, there is an ongoing debate whether PPIs are overprescribed, putting certain populations at unnecessary risk of side effects. In combination with the high incidence of osteoporotic fractures, the mean incidence of hip fractures alone between 1986 and 2005 was 957 per 100,000 women over the age of 65 per year, a small increase in risk suddenly has implications for a very large population. (Kopic & Geibel, 2013)

Other authors suggest that high risk of fractures occurs not only with PPIs, but also with other antacid drugs, such as histamine H2 receptor antagonists (H2RAs). (Grisso, y otros, 1997) (Corley, Kubo, Zhao, & Quesenberry, 2010)

It seems that an acidic environment in the stomach facilitates the release of ionized calcium from insoluble calcium salts, and the calcium solubilization is thought to be important for its absorption. Acid suppressants such as PPIs increase gastric pH, which may decrease calcium absorption and bone density, finally leading to an increased risk of fractures. (Ito & Jensen, 2010)

Studies with calcium carbonate, labeled with $^{45}Ca$, have shown a significant reduction of calcium absorption in patients taking PPIs, compared to patients taking placebo. (O'Connell, Madden, Murray, Heaney, & Kerzner, 2005) This behavior suggests that calcium carbonate is not the most appropriate option to supplement calcium in chronic users of antacid medication, especially PPIs.

However, calcium is not the only nutrient whose absorption can be reduced by the use of PPIs; several studies have shown that the bioavailability of other minerals, like magnesium and iron, and vitamins like cobalamin (vitamin B12), are also affected by the reduced gastric acid secretion. (Ito & Jensen, 2010)

## 2.2. Magnesium

From the first case report in which hypomagnesemia was associated to the use of PPIs in 2006, (Epstein, McGrath, & Law, 2006) several analogous cases have been reported in scientific and academic journals. From 2006 to 2010, at least 8 additional reports were published, all of them describing patients with chronic PPIs exposure, presenting with symptoms characteristic of hypomagnesemia. (Metz, Sostek, Ruszniewski, & Forsmark, 2007), (Cundy, 2008), (Shabajee, Lamb, Sturgess, & Sumathipala, 2008), (Broeren, Geerdink, Vader, & van den Wall Bake, 2009), (Kuipers, Thang, & Arntzenius, 2009), (Hoorn, van der Hoek, de Man, Kuipers, & al., 2010), (Mackay & Bladon, 2010), (Francois, Levy-Bohbot, Caron, & Durlach, 2008) All this information allowed us to confirm the relationship between regular use of PPIs and low serum magnesium levels.

Based on 8 of the 9 reports mentioned above, in 2011 the U.S. Food and Drug Administration (FDA) released a safety announcement with the title: "Low magnesium levels can be associated with long-term use of Proton Pump Inhibitor drugs (PPIs)." (U.S. Food and Drug Administration (FDA), 2011) In this announcement, FDA warns that low serum magnesium levels can result in serious adverse events including muscle spasm (tetany), irregular heartbeat (arrhythmias), and convulsions (seizures); however, patients do not always have these symptoms. They also highlight that treatment of hypomagnesemia generally requires magnesium supplements.

As a result of FDA announcement, the labels of all prescription PPIs marketed in the U.S. were revised to include new safety information:

*"Hypomagnesemia has been reported rarely with prolonged treatment with PPIs."* (AstraZeneca Pharmaceuticals LP, 2012)

*"Hypomagnesemia, symptomatic and asymptomatic, has been reported rarely in patients treated with PPIs for at least three months, in most cases after a year of therapy. Serious adverse events include tetany, arrhythmias, and seizures. In most patients, treatment of hypomagnesemia required magnesium replacement and discontinuation of the PPI."* (AstraZeneca Pharmaceuticals LP, 2012)

Following the safety announcement of FDA in 2011, 3 larger observational studies also supported this association in both inpatient and outpatient populations. (Gau, Yang, Chen, & Kao, 2012), (Luk, Parsons, Lee, & Hughes, 2013), (Zipursky, et al., 2014)

## 2.3. Iron

Numerous animal, as well as human clinical trials support the conclusion that the absorption of iron is markedly increased by gastric acidity. Several clinical conditions associated with achlorhydria/ hypochlorhydria (atrophic gastritis, pernicious anemia, gastric resections, vagotomy) have been shown to be associated with decreased iron absorption and/or iron-deficiency anemia. In rats, PPI treatment decreased iron absorption in animals taking a low iron diet. (Ito & Jensen, 2010)

In 2011 a study conducted by the Department of Medicine of the Michigan State University showed that chronic use of PPIs significantly decreases several hematologic indices. (Sarzynski, Puttarajappa, Xie, Grover, & Laird-Fick, 2011) The study was a retrospective cohort of 98 adult patients who received PPI therapy for at least one year. The authors compared the change in hematologic indices among patients receiving PPI therapy with matched controls. All hematologic indices decreased significantly from baseline: hemoglobin (-0.19 g/dl), hematocrit (-0.63%) and mean corpuscular volume (-0.49 fL). After adjustment for confounders, the odds ratio of decreasing hemoglobin by 1.0 g/dL while on chronic PPI therapy was 5.03, while the odds ratio of decreasing hematocrit by 3% was 5.46 both significant at 0.001 level.

(Shikata, et al., 2015) reported an investigation in which the clinical characteristics of 278 outpatients who received blood test including complete blood count and serum creatinine concentration were analyzed. The frequency of anemia was 51% in patients receiving PPI and 19% in those not receiving PPI (chi-squared test, $P<0.001$). Among these patients, the change in Hb after the initiation of PPI in 36 patients within 1 year before and within 1 year after the initiation of PPI was also investigated. Mean decrease in Hb after the initiation of PPI was 0.38±0.87 g/dl (95% confidence interval: –0.67 to –0.09 g/dl).

In 2016 another study published by the International Journal of Cardiology confirmed that proton pump inhibitors may affect red blood count indexes. (Boban, et al., 2016) The study involved 604 heart treatment patients who were scheduled for cardiac rehab two to six months after their

treatment. Of those, 294 were using proton pump inhibitors. Patients who had been diagnosed with atrial fibrillation were more likely to be taking the drugs, while those who had suffered a recent heart attack or who were obese were less likely. The analysis revealed that patients taking proton pump inhibitors were six-times more likely to suffer from anemia. They also had a worsening metabolic profile, which could have been the result of less frequent use of ACE inhibitors or statins.

More recently, in March 2017, a community-based case-control study was published in Gastroenterology. (Lam, Schneider, Quesenberry, & Corley, 2017) the association between acid suppressing medication use and the subsequent risk of iron deficiency was evaluated in 77.046 cases patients with new iron deficiency diagnoses (January 1999 - December 2013) and 398.314 controls. Both, PPIs and H2RAs consumption for more than 2 years were associated with an increased subsequent risk for iron deficiency. The odds ratio was 2.49; 95% confidence interval, 2.35 - 2.64 for PPIs and 1.58; 95% CI, 1.46 - 1.71 for H2RAs.

In addition to the studies mentioned above, several case reports of iron deficiency caused by regular use of PPIs have been published in the scientific literature e.g. (Hashimoto, Matsuda, & Chonan, 2014) (Dado, Loesch, & Jaganathan, 2017); however, the study published by (Lam, Schneider, Quesenberry, & Corley, 2017) is one of the most significant, because of the large number of patients involved, as well as the high odds ratio obtained.

## 2.4. Vitamin B12

It is well established that gastric acid secretion is needed for dietary Vitamin B12 absorption from foods. In short-term studies, various acid suppressants (PPIs and H2RAs) have been reported to decrease the absorption of vitamin B12 from foods, but not to decrease absorption of crystalline vitamin B12 which is not protein bound. (Ito & Jensen, 2010)

In a big case-control study within the Kaiser Permanente Northern California population, 25.956 patients having incident diagnoses of vitamin B12 deficiency, between January 1997 and June 2011, were compared with 184.199 patients without B12 deficiency. This study showed that previous and current gastric acid inhibitor use, was significantly associated with the presence of vitamin B12 deficiency. (Lam, Schneider, Zhao, & Corley, 2013)

The FDA-approved label for all PPIs declare: *"Daily treatment with any acid-suppressing medications over a long period of time may lead to malabsorption of cyanocobalamin (vitamin B-12) caused by hypo-or achlorhydria."*. (AstraZeneca Pharmaceuticals LP, 2012)

Monitoring and, in some cases, supplementation of these nutrients are advised. (Lee, 2012)

## 2.5. Intestinal flora

Malabsorption of nutrients is not the only negative consequence of the acid-reducing drugs. Hydrochloric acid plays an important role in the balance the gastrointestinal flora, so the sustained increase in the gastric pH values can cause proliferation of several microbes.

The *Clostridium difficile* associated diarrhea is a well-known adverse effect of the PPIs. Diarrhea itself impairs the quality of life of affected patients, but beyond is a predisposing factor for collagenous colitis. (Shimura, et al., 2012)

The FDA approved label for all PPIs declare: *"Published observational studies suggest that PPI therapy may be associated with an increased risk of Clostridium difficile associated diarrhea…"* (AstraZeneca Pharmaceuticals LP, 2012)

*Candida* proliferation is another imbalance in the gastrointestinal flora which has been reported when PPIs are consumed. (Larner & R., 1992) (Chocarro Martínez, et al., 2000) (Goenka, et al., 1996)

When acid secretion is impaired, *H. pylori* colonizes the body of the stomach determining a corpus predominant gastritis. Studies confirmed a progression toward atrophic gastritis in *H. pylori* positive patients on long term omeprazole, compared to no risk in *H. pylori* negative patients, which increases the risk of gastric cancer. That's why *H. pylori* has been classified as a carcinogen by World Health Organization. (Lee, 2012)

# 3. NUTRITIONAL DEFICIENCY MECHANISM

## 3.1. Calcium

A variety of reasons could theoretically account for the observation that PPIs increase the likelihood of fractures. The most prominent hypothesis assumes that the reduced acidity in the stomach impairs the intestinal absorption of dietary calcium.

An acidic environment in the stomach facilitates the release and solubilization of ionized calcium from insoluble calcium salts, which is the form how it is usually present in foods and dietary supplements. On the other hand, calcium solubilization is thought to be important for its absorption. Acid suppressants such as PPIs, as well as other antacid medications, increase gastric pH, which may decrease calcium absorption and bone density, finally leading to an increased risk of fractures.

For most of the calcium salts, the solubility decreases as the pH increases. (Goss, Lemons, Kerstetter, & Bogner, 2007) As shown in figure 1, the dissolution and subsequent absorption of this nutrient can be easily affected with the gastric pH modification.

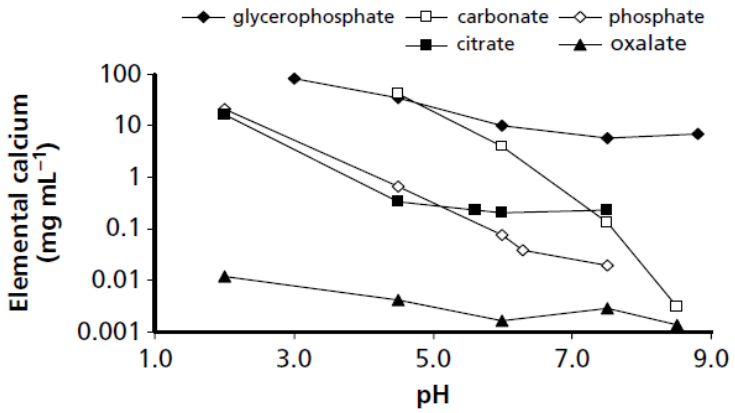


*Figure 1. Log solubility plot of calcium salts (median value) in aqueous solution open to the atmosphere.*

A study performed at the Wayne State University evaluated the effect of proton pump inhibitors on calcium carbonate absorption. Subjects ingested omeprazole 20 mg or placebo every morning for 7 days. After an overnight fast, each subject ingested two capsules containing radiolabeled calcium carbonate plus vitamin D. Calcium absorption was calculated as the fraction of the calcium tracer dose absorbed after 5 hours.

The results, plotted in figure 2, shown a significant reduction of the calcium absorption from calcium carbonate, in patients taking omeprazole. (O'Connell, Madden, Murray, Heaney, & Kerzner, 2005)

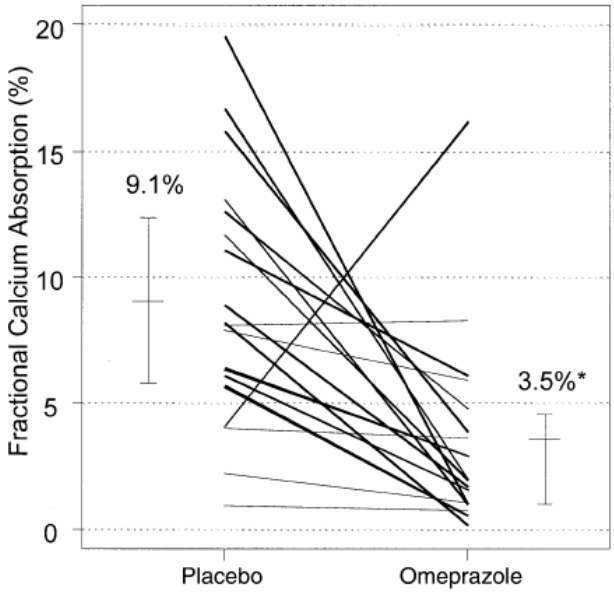


*Figure 2. Fractional calcium absorption for each subject (N = 18) after 1 week of placebo and omeprazole 20 mg. The 25th to 75th percentile bars and means are depicted for each treatment period. *P=0.003 for the difference between omeprazole and placebo.*

## 3.2. Magnesium

At present the mechanism(s) of the PPI induced hypomagnesemia is not clear. One study tested the hypothesis that it occurs in poor metabolizers of PPI, but that was not the case. It was concluded in one study that it is not specific to a given PPI, but is a generic problem with the PPI class of drugs, because it recurs even when PPIs are changed from one to the other.

A recent publication from the World Journal of Nephrology proposes an interesting hypothesis. (William & Danziger, 2016)

According to the authors, the available information suggested that PPIs-induced hypomagnesemia (PPIH) is not caused by renal magnesium wasting, since lower urinary magnesium has been reported in PPIs users. On the other hand, the statistical models controlled for other measures of dietary intake suggests decreased intestinal magnesium uptake. These clinical observations are supported by more recent mechanistic studies, which have focused on the potential effect of PPIs on the TRMP6 transporter, the major pathway of intestinal magnesium absorption.

Since the activity of TRMP6 is pH-dependent, being higher at an acidic milieu, the reduction of the gastric acid production caused by PPIs could potentially decrease its activity, reducing this way the intestinal uptake of magnesium, as shown in figure 3.

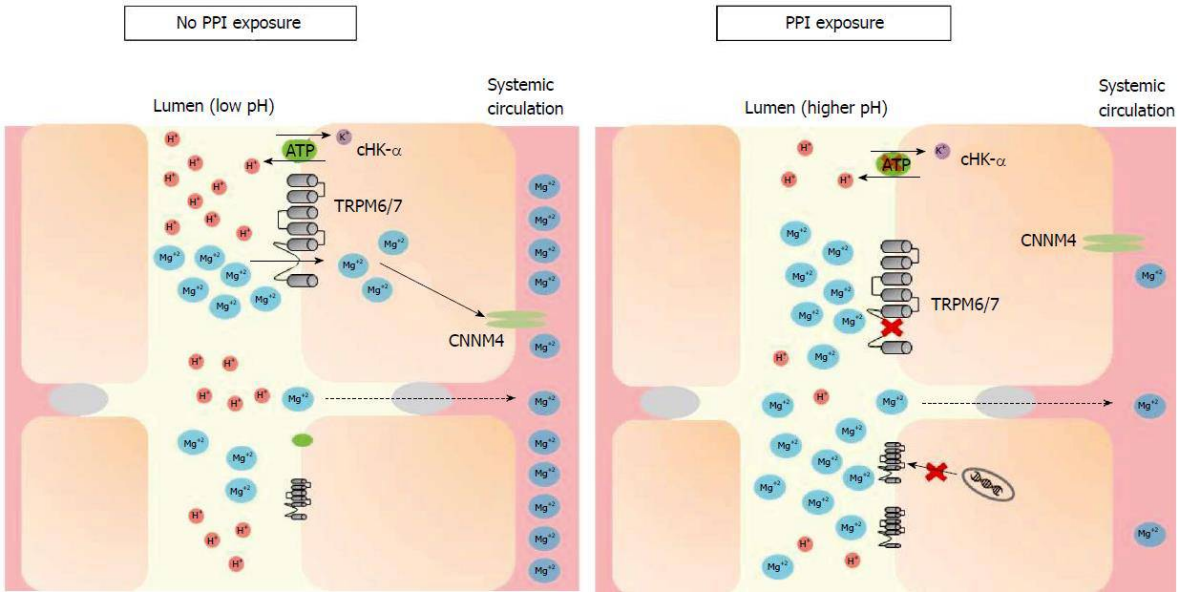


*Figure 3. Under conditions of magnesium deficiency, proton-pump inhibitors may inhibit magnesium absorption by increasing the pH of the intestinal lumen, through both gastric and non-gastric antagonism of the H+-K+ ATPase pump ("proton pump").*

As a final conclusion, authors warned that prospective studies that carefully control for nutritional intake among PPI users and accurately measure total body magnesium are needed to help determine causality of the association of PPI use and hypomagnesemia.

## 3.3. Iron

Dietary iron is present in food as either non-heme (66%) or heme iron (32%), and the non-heme iron's absorption is markedly improved by gastric acid. Gastric acid helps the non-heme iron containing food sources to dissociate and solubilize the iron salts, which allows the formation of complexes with ascorbate, sugars and amines facilitating this way its absorption. (Ito & Jensen, 2010)

It is well known that PPIs can increase the gastric pH through their inhibition of the H+/K+-ATPase pump on the parietal cell, thereby making the gastric environment more alkaline. The reduced form of iron ($Fe^{2+}$; ferrous iron) is what gets absorbed in the duodenum. The process of reducing iron to the ferrous form is influenced by the acidity or pH of the luminal contents. Proton pump inhibitors can increase the pH thereby causing the oxidation of some of the ferrous iron ($Fe^{2+}$) to ferric iron ($Fe^{3+}$), may negatively impact the bioavailability of iron in the duodenum. Once normal peristalsis moves the iron past this part of the intestine, it will not be absorbed. (Busti & Herrington, 2015)

## 3.4. Vitamin B12

Vitamin B12 is an essential nutrient that must be acquired from the diet. It's present in foods bound to protein, and the presence of gastric acid is required for the peptic enzymes, mainly pepsin, to cleave the vitamin B12 from the ingested dietary protein, allowing its reassociation with intrinsic factor (IF) and eventual absorption in the terminal ileum. (Ito & Jensen, 2010)

Thus, PPIs and H2RAs, which suppress the production of gastric acid, may lead to malabsorption of vitamin B12.

## 3.5. Intestinal flora

The GI tract has three different defense mechanisms: integrity of the membranes and mucous layer, GI microflora, and gastric acidity. The stomach is normally free of bacteria due to its acidity. Acid suppressive therapy may cause bacterial overgrowth in the upper GI tract and gastric colonization by several pathogen microorganisms.

# 4. RELATED ADVERSE EFFECTS

## 4.1. Calcium

As mentioned in the chapter number 2, long-term PPI therapy (a year or longer), particularly at high doses, is associated with an increased risk of hip, wrist and spine fracture.

## 4.2. Magnesium

Severe hypomagnesemia, as presented in patients who had been taking PPIs a mean of 8.3 years, can cause: fatigue, unsteadiness, paresthesia, muscle spasm (tetany), convulsions (seizures), irregular heartbeat (arrhythmias), hospitalizations. (Ito & Jensen, 2010)

## 4.3. Iron

In some studies of patients with long-term PPI use evidence for decreased iron absorption has been found which was attributed to the PPI (decreased ferritin, hemoglobin and iron levels, iron deficiency anemia).

## 4.4. Vitamin B12

Left untreated, vitamin B12 deficiency can lead to dementia, neurologic damage, anemia, and other complications, which may be irreversible.

The evidence about the relationship between PPIs and dementia is still controversial; however, there is a hypothesis suggesting that this relationship is mediated by a vitamin B12 deficiency. This hypothesis is based in two facts:

First fact: It was clearly established by (Lam, Schneider, Zhao, & Corley, 2013) that continuous use of PPIs as well as other acid suppressant drugs, is significantly associated with the presence of vitamin B12 deficiency.

Second fact: Several studies and systematic reviews have shown that vitamin B12 deficiency may increase the risk of suffering cognitive problems and dementia.

Although previous studies failed in showing a relationship between serum vitamin B12 levels and cognitive decline, at least 5 studies using newer biomarkers of vitamin B12 status (methylmalonic acid and holotranscobalamin (holoTC)) actually showed an association between poor vitamin B12

status and an increased risk of cognitive decline or dementia diagnosis. (Vogiatzoglou, et al., 2013) (O'Leary, Allman-Farinelli, & Samman, 2012)

## 4.5. Intestinal flora

Diarrhea is the most common adverse event with long term PPI use (3.7-4.1%). Diarrhea is associated to the colonization of *Clostridium difficile* and other pathogenic bacteria, throughout the gastrointestinal tract.

Colonization of upper gastrointestinal tract is also possible. Esophageal candidiasis is another common side effect of long term use of PPIs. (Larner & R., 1992) (Chocarro Martínez, et al., 2000) (Goenka, et al., 1996)

Colonization of *H. pylori* in the body of the stomach is definitely a concern after the long-term use of PPIs, because this colonization can determine a corpus predominant gastritis or atrophic gastritis, which are both risk factors for the development of adenocarcinoma.

## 5. FORMULA

| SUPPLEMENT | AMOUNT PER SERVING | % DAILY VALUE | RATIONALE |
|---|---|---|---|
| Calcium (as calcium citrate malate) | 250 mg | 25 % | Calcium malabsorption (Absorbable form of calcium) |
| Vitamin D3 (as cholecalciferol) | 800 IU | 200 % | Calcium malabsorption (Guarantees calcium absorption) |
| Vitamin K (as menaquinone-7) | 100 µg | 125 % | Calcium malabsorption (Guarantees calcium fixation) |
| Magnesium (as magnesium oxide) | 100 mg | 25 % | Hypomagnesemia |
| Iron (as Ferrochel® ferrous bisglycinate) | 18 mg | 100 % | Iron malabsorption (Absorbable form of iron) |
| Vitamin B12 (as methylcobalamine) | 100 µg | 1667 % | Vitamin B12 malabsorption (Absorbable form of vitamin B12) |
| Proprietary blend | 150 mg | * | Intestinal flora imbalance |
| *Lactobacillus acidophilus* | | | (Probiotic) |
| *Lactobacillus casei* | | | (Probiotic) |
| FOS (fructooligosaccharides) | | | (Prebiotic) |
| Inulin | | | (Prebiotic) |

*Daily value not stablished

Serving size: 2 capsules
Servings per container: 30 servings or 60 capsules

## 6. FARMACEUTICAL DOSAGE FORM AND PACKAGING MATERIAL

"00" elongated two-piece hard gelatin white/white capsule, where each capsule contains 523 mg of calcium citrate malate, equivalent to 125 mg of calcium + 10 mcg of cholecalciferol, equivalent to 400 IU of vitamin D3 + 50 µg of Menaquinone-7 + 83 mg of magnesium oxide, equivalent to 50 mg of magnesium + 45 mg of iron bisglycinate, equivalent to 9 mg of iron + 50 µg of vitamin B12 + 75 mg of a proprietary blend containing *Lactobacillus acidophilus* + *Lactobacillus casei* + FOS (fructooligosaccharides) + inulin.

60 capsules bottled in a 150 CC white HDPE bottle / 38mm white ribbed CRC cap with heat seal / 4 color process label / cotton / desiccant.

# 7. RATIONALE OF THE COMPONENTS

## 7.1. Calcium (as citrate malate) + Vitamin D3

Since the dissolution and subsequent absorption of calcium, from conventional sources, is affected by the suppression of gastric acid secretion, a special form of calcium, with pH-independent absorption properties, is required for those patients who consume antacid medication.

| Ca salt | Potential sensory characteristics | % Solubility Ca salt in $H_2O$ | Temperature (° C) |
|---|---|---|---|
| Calcium chloride·$6H_2O$ | Bitter notes, tissue irritant | 74 | 20 |
| Calcium lactate Gluconate | Clean tasting | 40 | 20 |
| Calcium acetate | Vinegary taste | 40 | 0 |
| | | 30–34 | 100 |
| Calcium formate | N/A | 16 | 20 |
| Calcium lactate·$5H_2O$ | Neutral, bitter at high levels | 9 | 20 |
| Calcium gluconate·$H_2O$ | Neutral taste | 3 | 20 |
| Calcium fumarate·$3H_2O$ | Neutral to slight fruity flavor in juice-based products | 1.22 | 20 |
| Calcium citrate malate (6:2:3) | Neutral taste and flavor | 1.1 | 25 |
| Calcium malate·$3H_2O$ | Slight sourness | 0.31–0.4 | 25 |
| Calcium citrate | Bitter notes and tangy/sour flavor at high concentrations | 0.096 | 25 |
| Calcium hydroxide | Slight bitter, alkaline taste | 0.1 | 20 |
| Calcium oxide | Alkaline, bitter taste | 0.1 | 25 |
| Dicalcium phosphate | Chalky mouthfeel at neutral pH | 0.02 | 25 |
| Tricalcium phosphate | gritty in liquids | 0.002 | 25 |
| Calcium carbonate | Soapy flavor, lemony taste | 0.0014–0.0056 | 25 |
| Calcium oxalate | N/A | 0.00067 | 20 |

*Figure 4. Solubility and sensory characteristics of various calcium sources.*

Calcium citrate malate is a water-soluble calcium form. It is the calcium salt of citric acid and malic acid with variable composition. It is purported to be highly bioavailable. Calcium citrate malate is nine times more soluble than either citrate or malate alone. (Heaney, 2001) Also, as shown in figure 4, calcium citrate malate is between 200 and 780 more soluble than calcium carbonate. (Reinwald, Weaver, & Kester, 2008)

Calcium citrate malate's bioavailability stems from its water-solubility and its method of dissolution. When dissolved, it releases calcium ions and a calcium-citrate complex. Calcium ions are absorbed directly into intestinal cells, and the citrate complex enters the body through paracellular absorption.

The chemistry of CCM makes it a particularly beneficial calcium source for individuals with hypochlorydia or achlorydia. (Reinwald, Weaver, & Kester, 2008)

The amount per serving of 250 mg for calcium in PROTON Support was compared to the amounts in the total population of dietary supplements in the U.S. The comparison was performed by using the Dietary Supplement Label Database of the National Institute of Health, and the findings showed that the content of calcium in PROTON Support is above the median. (See figure 5) (NIH Office of Dietary Supplements, 2015)

The amount per serving of 800 IU for vitamin D3 in PROTON Support was also compared to the amounts in the total population of dietary supplements in the U.S. The findings showed that the content of vitamin D3 in PROTON Support is above the median. (See figure 6) (NIH Office of Dietary Supplements, 2015)

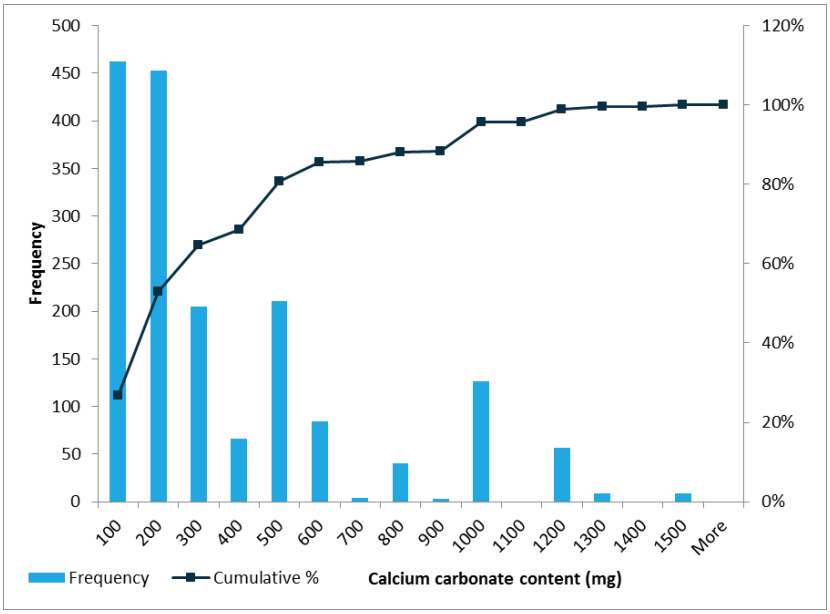


*Figure 5. Calcium (as calcium carbonate) content in dietary supplements in the U.S. (unidose dosage forms)*

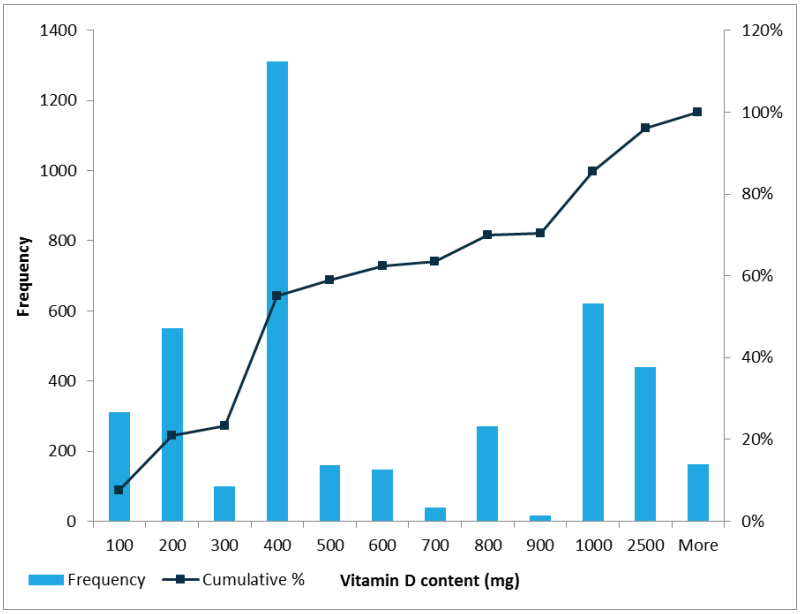


*Figure 6. Vitamin D content in dietary supplements in the U.S. (unidose dosage forms)*

## 7.2. Vitamin K2 (Menaquinone-7)

Recently, vitamin K2 has shown to work synergistically with a number of other nutrients, including calcium and vitamin D.

Vitamin K is actually a group of fat-soluble vitamins. Of the two main ones, K1 and K2, the one receiving the most attention is K1, which is very easy to get through the diet. Vitamin K2, also called menaquinone, is made by the bacteria that line the gastrointestinal tract; K2 goes straight to the blood vessel walls, bones, and tissues other than the liver.

Vitamin K2 can be broken into two additional categories, called MK-4 (menaquinone-4) and MK-7 (menaquinone-7). MK-7 is a longer-chain forms found in fermented foods. There's a variety of these long-chain forms but the most common one is MK-7. This is the one which is desirable to find in food supplements, because in a supplement form, the MK-4 products are actually synthetic. They are not derived from natural food products containing MK-4.

The MK-7 (long-chain, natural bacterial-derived vitamin K2) is obtained from a fermentation process, which offers a number of health advantages; it stays in the body longer, and It has a longer half-life, which means that it can be taken once a day in very convenient dosing.

When vitamin D is ingested, the body creates more vitamin K2-dependent proteins, the proteins that will move the calcium around. They have a lot of potential health benefits. But until the K2 comes in to activate those proteins, those benefits aren't realized. So, if somebody is taking vitamin

D, the body creates an increased demand for K2. Vitamin D and K2 work together to strengthen the bones.

Several studies have shown the relationship between calcium, vitamin D and vitamin K2. (Zittermann, 2001) (Flore, et al., 2013) (Gajic-Veljanoski, Bayoumi, Tomlinson, Khan, & Cheung, 2012) (Huang, et al., 2015)

Most of the studies were performed by using 100 µg of vitamin K2 per day. According to that, the amount per serving of vitamin K2 in PROTON Support is 100 µg.

The amount per serving of 100 µg for vitamin K2 in PROTON Support was compared to the amounts in the total population of dietary supplements in the U.S. The findings showed that the content of vitamin K2 in PROTON Support is above the median. (See figure 7) (NIH Office of Dietary Supplements, 2015)

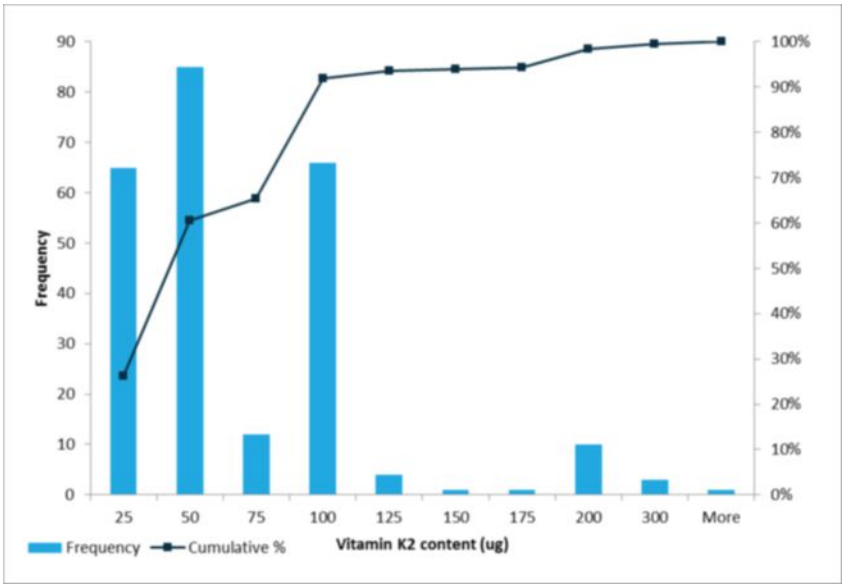


*Figure 7. Vitamin K2 content in dietary supplements in the U.S. (unidose dosage forms)*

## 7.3. Magnesium

Magnesium oxide is one of the most used forms of magnesium supplements. The content of magnesium in magnesium oxide is 5 times bigger when compared to magnesium citrate, which means that less total quantity of the salt is required in order to achieve the desired quantity of magnesium. This characteristic is very useful in this product in which big quantities of other compounds are required and the size of the capsule is limited.

The amount per serving of 100 mg for magnesium in PROTON Support was compared to the amounts in the total population of dietary supplements in the U.S. The findings showed that the content of magnesium in PROTON Support is above the median. (See figure 8) (NIH Office of Dietary Supplements, 2015)

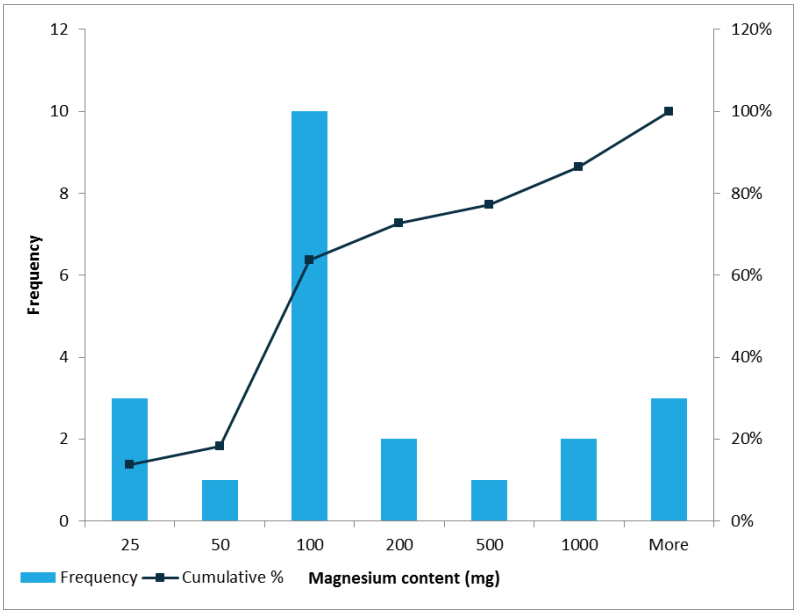


*Figure 8. Magnesium content in dietary supplements in the U.S. (unidose dosage forms)*

## 7.4. Iron (Ferrous bis-glycinate, Ferrochel®)

Since the dissolution and subsequent absorption of non-HEME iron is affected by the suppression of hydrochloric acid, a special form of iron, whose absorption is pH-independent, is required.

Iron bis-glycinate is a source if iron in which ferrous iron is reacted with glycinate to form bis-glycinate chelate, a non-electrically charged compound that is totally nutritionally functional. The absence of electrical charge, uncommon for an iron supplement, makes it less likely that Iron bis-glycinate can interfere with the absorption of other minerals such as calcium, vitamin E or vitamin C. Iron solubility of iron bis-glycinate chelate is not affected by pH changes between 2 to 6. This means that it travels unchanged trough the stomach, into the intestine, where it is absorbed and released for transportation throughout the body. (García-Casal & Layrisse, 2001)

Because iron bis-glycinate is a neutral, fully-reacted molecule, it doesn't break down in stomach acid and is delivered intact to the intestine, where it is easily absorbed. In other words, by using iron bys-glycinate the step in which the hydrochloric acid is required is avoided. iron bis-glycinate is bioavailable even under achlorhydria condition.

## 7.5. Vitamin B12

Vitamin B12 deficiency is relatively common, especially among older adults; it has potentially serious medical complications if undiagnosed. According to data from the National Health and Nutrition Examination Survey, 3.2% of adults older than 50 years are estimated to have low serum vitamin B12 levels. Thus, identifying and acting over modifiable risk factors for vitamin B12 deficiency is of significant public health importance. (Lam, Schneider, Zhao, & Corley, 2013)

Regarding the diet-sourced B12 vitamin, the presence of gastric acid is needed for the peptic enzymes, mainly pepsin, to cleave the vitamin B12 from the protein, allowing its reassociation with intrinsic factor (IF) and eventual absorption in the terminal ileum. The crystalline B12 vitamin does not need to be separated from proteins by the peptic enzymes, so hydrochloric acid is not required.

In short-term studies, various acid suppressants (H2RAs and PPIs) have been reported to decrease the absorption of vitamin B12 from foods, but not to decrease absorption of crystalline vitamin B12 which is not protein bound. (Ito & Jensen, 2010) According to that, crystalline methylcobalamin is an appropriate source of vitamin B12, for patients in risk of deficiency of this important nutrient, on account of PPIs or H2RAs consumption.

The amount per serving of 100 µg for vitamin B12 in PROTON Support was compared to the amounts in the total population of dietary supplements in the U.S. The findings showed that the content of vitamin B12 in PROTON Support is above the median. (See figure 9) (NIH Office of Dietary Supplements, 2015)

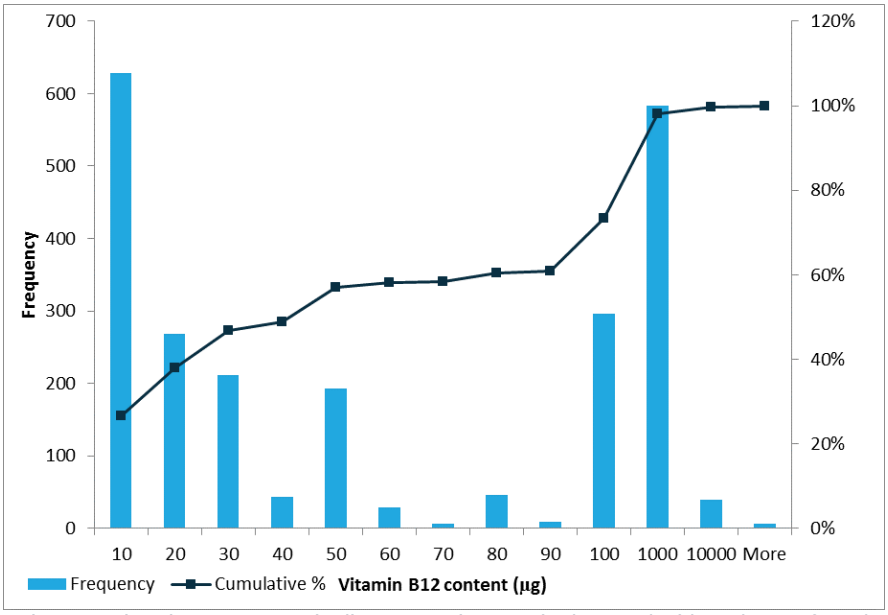


*Figure 9. Vitamin B12 content in dietary supplements in the U.S. (unidose dosage forms)*

### 7.6. *L. acidophilus + L. casei*

Probiotics or "friendly" bacteria may help maintain a balance in the digestive system between good and harmful bacteria such as *Clostridium difficile*. According to the Cochrane review, (Goldenberg, et al., 2013) probiotics have shown to be beneficial in the prevention of *Clostridium difficile* associated diarrhea. One of the most effective mixture of probiotics was *L. acidophilus* + *L. casei*.

In the same way in which probiotics help to suppress the *Clostridium difficile* infection, they can also avoid the *H. pylori* colonization, becoming itself into an adjuvant treatment in some gastric diseases.

The medical center of the University of Maryland recommends the use of dietary supplements containing 5 to 10 billion CFUs of *L. acidophilus,* as an adjuvant treatment in peptide ulcer. (University of Meryland Medical Center, 2015)

With a serving size of two capsules, the total amount of probiotics, in a daily treatment with PROTON Support, is 5 billion of *L. acidophilus* + 5 billion of *L. casei*, which agrees with the recommendation of the Maryland University, as well as the findings in the Cochrane review.

## 8. FACTS AND CLAIMS

### 8.1. Facts

- Long-term proton pump inhibitors therapy may be associated with an increased risk for osteoporosis-related fractures of the hip, wrist or spine.
- Daily long-term use of proton pump inhibitors may affect the absorption, or lead to a deficiency of magnesium, iron and vitamin B12.
- Proton pump inhibitors therapy may be associated with increased risk of Clostridium difficile associated diarrhea.

### 8.2. Claims

- Helps build strong bones & may help prevent osteoporosis.

- Helps maintain healthy levels of magnesium, iron and vitamin B12.
- Promotes a healthy digestive system.

## 9. PATENT PROTECTION

A patent application was submitted to the USPTO.

## 10. BIBLIOGRPHY

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