Effect of proton pump inhibitors on magnesium balance: is there a link to cardiovascular risk?

Laura Francesca Pisani[1], Elisabetta Filippi[2], Sara Vavassori[1], Nadia Munizio[1], Maurizio Vecchi[1,2], Luca Pastorelli[1,2]

[1] Gastroenterology Unit, IRCCS Policlinico San Donato, San Donato Milanese, Italy; [2] Department of Biomedical Sciences for Health, University of Milan, Milan, Italy

Correspondence: Luca Pastorelli. University of Milan, Department of Biomedical Sciences for Health. Piazza Malan, 20097, San Donato Milanese, Italy
<luca.pastorelli@unimi.it>

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Abstract. Magnesium (Mg^{2+}) is the second most copious element inside human cells and the fourth most abundant positively charged ion in the human body. It is of central importance for a broad variety of physiological processes, including intracellular signaling, neuronal excitability, muscle contraction, bone formation and enzyme activation. Its overall balance is tightly regulated by the concerted actions of the intestine, bones and kidneys. Disturbance of this balance can have serious consequences. Symptoms of hypomagnesaemia include tetany, seizures and cardiac arrhythmias, whereas hypermagnesaemia may cause cardiovascular and neuromuscular abnormalities. Drugs can interfere with Mg^{2+} homoeostasis in several ways, and proton-pump inhibitors (PPIs) have been associated with hypomagnesaemia. A better understanding of the molecular mechanisms underlying the adverse effects of these medications on Mg^{2+} balance will isuggest ideas for prevention and treatment, and might provide greater insight into Mg^{2+} homoeostasis.This review gives an overview of the influence of PPIs on Mg^{2+} homoeostasis and provides some understanding of the underlying physiological mechanisms. Moreover, we will discuss the potential link between PPI-induced changes in Mg^{2+} homeostasis, and the reported cardiovascular risk observed in long-term PPI users.

Key words: magnesium, proton pump inhibitors, hypomagnesaemia

Magnesium (Mg^{2+}) is integral to cellular and systemic human physiology, and the body's ability to function. Yet, this mineral is often overlooked compared to other cations such as calcium or iron. Mg^{2+} is the fourth most abundant element in the human body and most of it is stored in bones (50%) and soft tissues (47%) [1].

It is a critical cofactor in any reaction powered by ATP, so a deficiency of this ion may have dramatic effects on metabolism. Magnesium also acts as a calcium channel antagonist and plays a key role in the modulation of any activity governed by intracellular calcium concentration fluxes (e.g., muscle contraction, insulin release) [2].

Adult men contain approximately 24 g of magnesium, half of this being stored in mineralised form in bones. Almost all of the remainder is localised in the intracellular compartment, with only 1% of total body magnesium in the extracellular space [3]. Normal plasma magnesium concentrations range from 1.8 to 2.3 mg/dL. Circulating magnesium exists in three states: ionised magnesium (60% of total magnesium), protein bound (30%, mostly albumin [4]) and complexed to serum anions (10%) [5]: only ionised magnesium is physiologically active [6, 7].

Many clinical signs, symptoms and disease states can be attributed to altered Mg^{2+}

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homeostasis caused by an imbalance between intestinal absorption and renal excretion. Growing evidence suggests that many drugs can cause changes to this balance and, more specifically, they can lead to hypomagnesaemia [3, 7]. Remarkably, the proton-pump inhibitors (PPIs), which are widely used for several gastroenterological conditions, are listed among them.

Magnesium homeostasis

The absorption of magnesium from the gastrointestinal tract is not fully understood, although it happens predominantly in the proximal gastrointestinal tract via a non-saturable paracellular passive pathway, and in the distal segments via a saturable transcellular active pathway [8]. While an active transport process occurs in the colon and ileum, the rest of the small intestine uses passive absorption for magnesium. At low intraluminal concentrations, magnesium is absorbed primarily via the transcellular route, which involves the active transport of magnesium into the blood through the interior of the epithelial cells; the passive paracellular pathway comes into play as magnesium concentrations rise [8].

Magnesium enters the eukaryotic cell through a number of channels, including magnesium transporter 1 (MagT1), membrane magnesium transporter homologue (MMgT), solute carrier family 1 member 1 (SLC1-A1), transient receptor potential cation channel family M, member 6 and 7 (TRPM6 and TRPM7, respectively) [9, 10].

In the gastrointestinal tract, transcellular Mg^{2+} transport in the apical membrane is predominantly mediated by TRPM6 and TRPM7, two cation channels that form complexes at the cell surface [11]. TRPM6 resides in the colon and in the distal convoluted tubule of the nephron, while TRPM7, ubiquitous in most mammalian cells, is usually present in various parts of the small intestine. Luminal Mg^{2+} influx is mediated by TRPM6 and is transported across the cell to the basolateral membrane and extruded into the blood [10], whereas TRPM7 is involved in the apical transport of Mg^{2+} [12, 13] (*figure 1*).

Paracellular transport takes place via tight junction proteins from the claudin family, and is driven by the electrochemical gradient across the epithelium [14, 15] (*figure 1*); as such, paracellular transport is effective only when dietary intake of Mg^{2+} is adequate.

Once in the bloodstream, one-third of the circulating magnesium is bound to proteins, mainly albumin, or is complexed to anions, whereas two-thirds are in the free form [1]. In the kidney, around 80% of total serum magnesium is filtered by the glomeruli, and >95% of it beingreabsorbed along the nephron [15].

Proton-pump inhibitors (PPIs)

Proton-pump inhibitor drugs (e.g. omeprazole, esomeprazole, lansoprazole, pantoprazole and rabeprazole) are potent inhibitors of gastric acid secretion, and act by blocking the hydrogen–potassium adenosine triphosphatase enzyme system (the 'proton pump') of the gastric parietal cell. They are widely used for the treatment and prevention of dyspeptic symptoms associated with peptic ulcer disease, gastritis and oesophagitis.

First introduced in the late 1980s PPIs are the most potent inhibitors of gastric secretion available, with efficacy superior to histamine-2 receptor antagonists [16, 17].

Proton-pump inhibitors have become one of the most commonly prescribed class of drugs in primary and speciality care. Long-term, sometimes lifetime use is becoming increasingly common, even in the absence of appropriate indications, and there is growing concern for potential adverse effects from such long-term therapy [18]. Furthermore, since PPIs are available over-the-counter, many patients use them without prior medical advice.

PPI drugs belong to the class of benzimidazoles, which act by blocking the proton pump of the gastric parietal cells, reducing basal and stimulated acid secretion.

All PPIs have good oral bioavailability: 77% for pantoprazole, 80% to 90% for lansoprazole, and 89% for esomeprazole. Currently available benzimidazole-based PPIs have similar half-lives of 1-2 h. Their anti-secretory effect starts within an hour after administration, reaching maximum suppression of all the proton pumps in approximately 3-4 days [19].

All the PPIs are rapidly metabolised in the liver by CYP enzymes (mostly by CYP2C19 and 3A4), except for rabeprazole, which is metabolized through a non-enzymatic pathway, with formation of a thioester compound by sulphoxide reduction [20]. Because of the sensitivity of PPIs to CYP

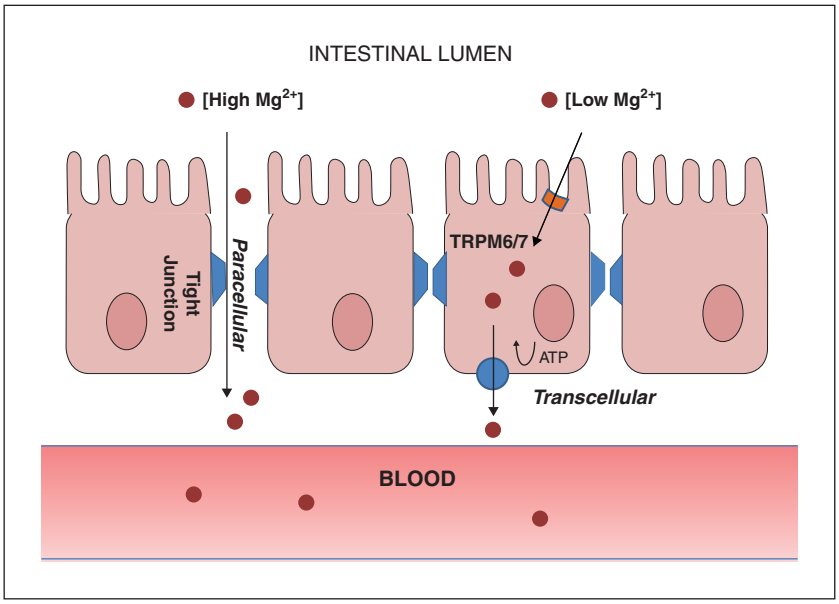

Figure 1. Mg^{2+} absorption in the gastrointestinal tract. Magnesium is passively absorbed via the paracellular route, when intraluminal magnesium concentrations are higher than plasmatic (left). On the other hand, at low intraluminal concentrations (right), magnesium is actively absorbed through transient potential melastatin-6 and -7 (TRPM6/7) channels within enterocytes (transcellular pathways), against a gradient.

enzymes, the pharmacokinetic profiles of PPIs are very different depending on the phenotypes of the metabolisers, and it is important to be aware of the pharmacological interactions of these drugs.

Acid suppression studies comparing omeprazole, lansoprazole, rabeprazole, and pantoprazole show equivalent efficacy. Most studies using standard doses have not shown a significant difference between the four PPIs for the healing of reflux oesophagitis or duodenal ulcer. Esomeprazole has stronger acid suppression, with a longer period of an intragastric pH greater than 4 [21].

Interaction between PPIs and magnesium homeostasis

Proton-pump inhibitors have an excellent safety profile and have become one of the most commonly prescribed class of drugs in primary and speciality care. The risk of minor adverse effects is low, occurring at a rate of 1-3% [18]. Each of the adverse effects (reported in *table 2*) is biologically possible, but, based on current evidence, we can say that the benefits of PPI use greatly outweigh the risk in most patients.

Nutritional deficiencies, although rare, are among the most common and relevant effects of PPIs, and they include vitamin B12 deficiency, iron deficiency and hypomagnesaemia. All PPI-induced nutritional deficiencies are due to the impaired intestinal absorption of micronutrients. In fact, these drugs may alter iron and vitamin B12 absorption by suppressing gastric acidity, necessary for physiological absorption of non-haeme iron and for the early steps of vitamin B12 absorption [18]. Hypomagnesaemia is very rare and usually remains undetected, but it may be triggered by PPI therapy leading to severe adverse reaction. Hypomagnesaemia associated with PPIs (PPIH) can cause a range of symptoms of varying incidence, including tremor of the extremities, convulsions (40%), muscle cramps and spasms (20%), weakness and lethargy (30%), tetany (17%), loss of consciousness, numbness, anxiety, hallucinations, agitation (20%), dizziness and nausea (36%), carpopedal spasm associated with hypoparathyroidism and hypocalcaemia, signs

Table 1. Manifestation of hypomagnesaemia.

Neuromuscular	Muscle weakness Muscle twitching and tremor Positive Chvostek's and Trousseau's sign Paraesthesia Tetany Vertical nystagmus Delirium Hyperreflexia Convulsions Coma	[3, 15, 60]
Cardiac	Premature ventricular contraction Prolongation of PR and QT intervals T-wave flattering Atrial and ventricular arrhythmias Myocardial infarction	[3, 45, 61]
Metabolic	Hypocalcaemia Hypokalaemia	[15, 61-63]

Table 2. The main adverse effects of proton-pump inhibitors.

		Theoretical mechanism	**References**
Nutritional deficiencies		Due to impaired intestinal absorption: PPIs suppress the gastric acidity necessary for physiological absorption of micronutrients, such as vitamin B12, iron and magnesium.	[18]
Risk of fractures		Long-term PPI use in some patients with osteoporosis or other risk factors for fracture, such as steroid use, may increase the risk of hip, wrist and spine fractures.	[64]
Infections	Community acquired pneumonia	Overgrowth of non-*H. pylori* bacteria in the digestive tract that is associated with pulmonary micro-aspiration and lung colonisation Impairment of neutrophil function and NK cell activity	[65]
	Enteric infections	Alterations in gastrointestinal microflora Increased risk of nosocomial *C. difficile*-associated diarrhoea	[66]
Hypergastrinaemia		Due to the trophic effects of gastrin, PPI use may increase the risk of fundic gland polyps (benign). No clinical evidence of relationship with gastric cancer, gastric carcinoids or colorectal cancer.	[18]
Interactions		PPIs inhibit components of the hepatic cytochrome P450 system, notably CYP2C19, CYP3A4, and CYP11A2. The most commonly reported interactions are with vitamin K antagonists and clopidogrel.	[67, 68]

such as Trousseau and Chvostek sign, QT prolongation, ataxia, concomitant hypokalaemia with electrocardiogram (ECG) changes and arrhythmias (30%). Tetany or neuromuscular irritability can be related to co-existent hypomagnesaemia and hypocalcaemia [22]. *Table 1* summarizes the main effects of hypomagnesaemia.

Epstein *et al*. provided the first description in 2006, reporting two cases of hypomagnesaemic hypoparathyroidism associated with the use of proton-pump inhibitors, in which patients presented with carpopedal and truncal spasms in association with severe hypomagnesaemia and hypocalcaemia, without an appropriate increase in the level of parathyroid hormone. In both cases the withdrawal of the drug (omeprazole) promptly normalised the metabolic abnormalities: the reintroduction of another PPI (esomeprazole) rapidly produce a new drop in magnesium levels [23]. Several other reports reiterated this initial description, all of them confirming the resolution of the clinical and biochemical alterations after PPI withdrawal; on the other hand, oral magnesium supplementation was often described as not effective or only partially effective in restoring magnesium serum levels. The occurrence of the hypomagnesaemia appeared to be linked to the pharmacological class, but not to a specific

molecule; in fact, this side effect was reported for omeprazole, esomeprazole, rapeprazole, and pantoprazole.

Interestingly, it has been demonstrated that hypomagnesaemic patients also display low urinary calcium and magnesium excretion; as such, this indicates that PPIs do not provoke magnesium renal loss, but it is likely that they interfere with intestinal magnesium absorption. It might be reasonable to hypothesise that malabsorption of magnesium is related to changes in gastric pH, as is the case for other micronutrient deficiencies (i.e. PPI-induced iron deficiency), however, this appears not to be the case; in fact, in these case series, when hypomagnesaemic patients were shifted to H_2-receptor antagonist (H2RA), such as ranitidine, magnesium levels progressively recovered. Thus, as will be discussed later, additional mechanisms should be taken into account to explain this phenomenon.

In addition to the aforementioned case reports, more recent, population-based cohort studies, overall, confirm the association between PPI use and the risk of hypomagnesaemia in the general population. According to Kieboom's study, which was performed prospectively in a cohort of 9818 patients from the general population, the risk of hypomagnesaemia was further increased when PPI use was prolonged (>six months) or combined with the use of loop diuretics [24]. In contrast to other reports, this cohort study found a similar, albeit slightly lower, risk in patients treated with H2RA compared to PPI-treated patients [24]. Indeed, larger studies involving a 95,205-subject cohort in a community setting [25], 1189 haemodialysis patients [26], and 1830 hospitalised patients [27], found no association between H2RA use and low serum magnesium levels, while confirming the increased risk of hypomagnesaemia in PPI-users.

Recent large cohort studies have also tried to provide estimates of the prevalence of hypomagnesaemia in patients taking PPIs. According to the setting and the study, it varies considerably, ranging from 0.4% in patients undergoing percutaneous coronary intervention [28], to 24% in a tertiary emergency department [29], and up to 79% in a cross-sectional study of haemodialysis patients [30]. Interestingly, Park's paper found no differences in serum magnesium levels between PPI-users and non-PPI-users [28]; although this finding is unexpected, several confounding factors, such as patients' medications, lifestyle, comorbidity and genetic background should be taken into account when evaluating these data.

As such, with the exception of Park's work, data in literature confirm the effect of PPIs in inducing hypomagnesaemia, even though the variable prevalence does not suggest whether or not this side effect affects only genetically susceptible subjects.

Taken together, we can assume that PPIs have a class effect in the aetiology of hypomagnesaemia. This is supported by the observation that the patients reported were taking different drugs; besides, the substitution of one PPI for another, results in a recurrence of symptoms of PPIH and electrolyte abnormalities [31].

In the majority of the symptomatic cases reported, the patients were severely magnesium-depleted and had vigorous renal magnesium retention [32-34]; accordingly, William *et al*. measured urinary magnesium in 278 consecutive ambulatory patients evaluated for nephrolithiasis. They found that PPI users displayed lower daily urinary magnesium [35], suggesting reduced intestinal magnesium absorption, although in no study were there obvious signs of generalised malabsorption

Several hypotheses have been developed to explain the mechanism of PPIH. It could be speculated that PPI drugs might affect tight junction function in the paracellular pathway, either directly or because of intestinal pH changes; alternatively, they might affect TRPM6 channel function in the saturable transcellular active pathway [33].

Cundy and Dissanayake noticed that high-dose magnesium supplements partially corrected PPIH. Therefore, they suggested that passive magnesium transport was intact and that PPI therapy affected the active transport pathway [32]. In fact, according to Bai *et al*., by changing the intestinal luminal pH, PPIs may induce defective functioning of the active transport channels TRPM 6 and -7 [36].

On the other hand, it has also been suggested that PPIs might impair the paracellular transport of magnesium by altering tight junction function, given the increase in intestinal permeability, detected after PPI administration [37]; alternatively, Thongon *et al*. suggested that omeprazole inhibits passive intestinal Mg^{2+} absorption, because paracellular cation pores have a greater affinity for Mg at low pH [38].

Given the fact that PPIH occurs only in a subset of patients, Hoorn *et al*. tested the hypothesis that

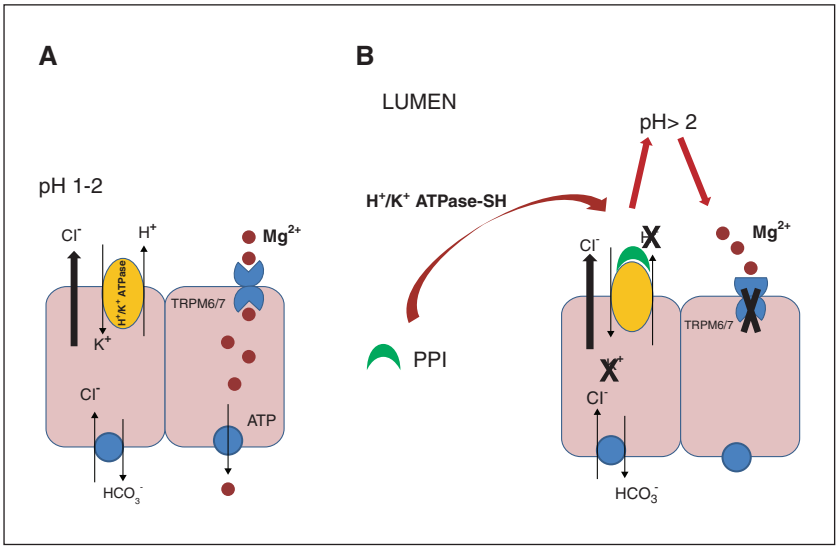

Figure 2. Possible mechanism by which proton-pump inhibitors interfere with the Mg^{2+} balance. A) Physiology of gastric acid secretion and absorption of magnesium via a saturable transcellular active pathway. **B)** The PPI blocks the hydrogen-potassium adenosine triphosphatase enzyme system (H+/K+-ATPase-SH) of the gastrointestinal epithelial cells, reducing the basal acid secretion. The consequent pH increase leads to conformational and functional changes in the transient potential melastatin-6 and -7 (TRPM6/7) channels.

PPI-induced hypomagnesaemia occurs in "poor metabolisers" of PPIs (with a deficit in CYP2C19), but found this not to be the case [28]; however, it raised the possibility that genetic factors result in increased susceptibility to PPI-induced hypomagnesaemia. Mutations in TRPM6 or impaired TRPM protein function after acid suppression have been proposed as a potential mechanism for hypomagnesaemia [32-34, 39]. This issue remains open, and it is not clear whether PPI-induced hypomagnesaemia is an idiosyncratic reaction, or contributed to by mutations of TRPM6 and -7 or other genetic factors [40].

In conclusion, the mechanism of PPIH has yet to be elucidated, but impaired intestinal absorption through PPI inhibition of paracellular passive transporter channels or transcellular active transporter channels, appears pivotal [41] (*figure 2*).

PPI and cardiovascular risk: is magnesium implicated?

In recent years, PPIs have been associated with adverse clinical outcomes amongst clopidogrel users following acute coronary syndrome (ACS). For individuals with a history of ACS, PPIs appear to reduce the efficacy of this anti-platelet, probably interfering with the cytochrome P2C19-mediated activation of clopidogrel [42]. However, some studies have associated PPI use with adverse clinical outcomes in high risk cardiovascular populations, independent of clopidogrel use [43, 44]. A very recent study was performed through data mining of the pharmacovigilance database of Stanford Translational Research Database Environment. Authors queried over 16 million clinical documents involving 2.9 million subjects from the general population, and analysed the association between PPI usage and cardiovascular risk [45]. The study confirmed an association, although this was not present in H2RA users, and was no greater in patients taking clopidogrel [45]. Thus, the observed risk, rather than being caused by the co-administration of a PPI and clopidogrel, is probably due to some other, as yet unknown mechanism. Several hypotheses have been proposed, but remain to be proven. For instance, it is known that PPIs inhibit dimethylarginine dimethylaminohydrolase (DDAH), which is responsible for 80% of the clearance of asymmetric

dimethylarginine (ADMA) - an endogenous molecule known to block the enzymatic activity of nitric oxide synthase (NOS) [46]. In addition, it could be hypothesised that PPI-induced hypomagnesaemia may have a role in this setting. In fact, an ever-growing wealth of data demonstrates the pivotal functions of magnesium in maintaining cardiovascular homeostasis [47]. For examplee, magnesium possesses strong vasodilator effects [48, 49], reduces platelet activation [50], is anti-inflammatory for endothelial cells, and dampens the formation of free oxygen radicals [51, 52]. Similarly, hypomagnesaemia plays a role in the pathogenesis of atherosclerosis through the creation of a pro-atherogenic lipidic profile [53], increased vascular calcification [54], impairment of insulin activity [55, 56] and the promotion of vascular chronic inflammation [57]. Magnesium is also known to regulate cardiac electric activity, exerting direct effects on potassium and calcium channels [58]. Taken together, it is reasonable to assume that hypomagnesaemia is an important risk factor for cardiovascular events. Indeed, this association has been repeatedly demonstrated in clinical observations [58, 59]; thus, it is plausible and fascinating to hypothesise that the increase in cardiovascular risk observed in cohorts of PPI-users is, at least partially, due to magnesium deficiency. However, this hypothesis has yet to be tested in case-control investigations and mechanistic studies; indeed, planning the proper study design is a real challenge, given the rich co-morbidity presented by most chronic PPI-users and the variety of variables influencing both hypomagnesaemia and cardiovascular disease.

Conclusion

PPIs are not only the most potent inhibitors of gastric acid secretion available, they have an excellent safety profile.

The risk of minor adverse effects is low, occurring at a rate of 1–3%: the benefits of PPI use outweigh the risks in most patients.

PPIH is a rare, but increasingly recognised clinical phenomenon, requiring a high index of suspicion for diagnosis. With long-term use of PPIs, it is important that clinicians are aware of the potential development of PPIH and related presentations. These range from no symptoms at all to lethargy and leg cramping, to seizures and life-threatening arrhythmias. Short-term PPI use is not usually associated with hypomagnesaemia; onset usually occurs after five years of PPI use. Hypomagnesaemia induces endothelial dysfunction that could promote atherosclerosis. Theoretically, this could be one of the reasons for the observed relationship between PPI use and increased risk of major cardiovascular events.

Promoting the appropriate use of PPIs and avoiding long-term use particularly when therapy is not indicated, should be a priority, along with monitoring of long-term therapy with regular laboratory tests for magnesium levels and other nutrients/minerals whose absorption might be impaired in the case of longstanding PPI use. Prompt cessation of PPIs and magnesium replacement can reverse PPIH and prevent re-hospitalisation and complications.

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