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In vitro and *In vivo* Comparison of Marine Magnesium salts (Oxide, Citrate, Bisglycinate, and Citrate Malate) Absorption and their Activity on Inflammatory Response and Oxidative Stress

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Abstract

Although dietary magnesium intake is common, its low bioavailability contributes to widespread deficiency in modern populations. This study aims to evaluate the absorption and biological activities of four marine magnesium forms (oxide, citrate, citrate malate, and bisglycinate) for maximizing magnesium biological effectiveness. Absorption of marine organic magnesium compounds was compared to marine magnesium oxide by Caco-2 intestinal permeability assay and their *in vivo* bioavailability in mice. Marine organic magnesium compounds are significantly more effective than marine magnesium oxide in both absorption and bioavailability. Among them, bisglycinate achieved the highest peak absorption, while citrate and citrate malate provided high and stable absorption; notably, citrate malate offered the fastest uptake. Marine organic magnesium compounds exhibit significantly stronger anti-inflammatory and antioxidant activities than magnesium oxide, with bisglycinate and citrate malate showing better efficacy than citrate. These results demonstrate that marine organic magnesium compounds are significantly more effective in terms of both intestinal absorption and bioavailability compared to marine magnesium oxide and exhibit more robust anti-inflammatory and antioxidant activities. In clinical applications, marine organic magnesium forms may be the preferred options for individuals seeking efficient and prolonged absorption, and marine magnesium citrate malate could be also more suitable for cases requiring rapid magnesium absorption.

Keywords: Marine Magnesium salts; Absorption; Bioavailability; Inflammation; Oxidative stress

List of Abbreviations: Mg2+: Magnesium; TNFα : Tumor-necrosis factor alpha; IL-: Interleukin; NF-κB: Nuclear Factor kappa-light-chain-enhancer of activated B cells; CRP: C-reactive protein; ROS: Reactive oxygen species; TRPM: Transient Receptor Potential Melastatin; Caco-2: Human colorectal adenocarcinoma cells; DMEM: Dulbecco's modified Eagle's medium; HBSS; Hanks' balanced salt solution; EFS: Etablissement Français du Sang; PBMC: Human peripheral blood mononuclear cells; qRT-PCR: Quantitative reverse transcription polymerase chain reaction; GAPDH: Glyceraldehyde 3-phosphate dehydrogenase; COX2: Cyclooxygenase 2; TGF: Transforming growth factor; Nrf2: Nuclear factor erythroid 2-related factor 2; HMOX1: Heme-oxygenase 1; CAT: Catalase; GSR: Glutathione reductase; NOS2: inducible nitric oxide synthase; ARG1: Arginase 1; NADPH: Nicotinamide Adenine Dinucleotide Phosphate Hydrogen

Introduction

Magnesium (Mg2+) is an essential mineral nutrient, necessary for many biochemical reactions in the human body, including energy metabolism, protein and DNA synthesis, maintenance of the electrical potential of nervous and cardiac tissues, control of blood glucose, and regulation of blood pressure. This mineral is involved in over 300 enzymatic reactions, thus contributing to various physiological processes such as energy production, metabolism regulation, muscle contraction, nerve signal transmission, and bone health [1].

Magnesium also plays a crucial role in the regulation of inflammation and oxidative stress, two pathophysiological mechanisms involved in many chronic diseases. Studies have shown that adequate magnesium levels in the body are involved in controlling the expression of pro-inflammatory cytokines [2, 3]. A decrease of tumor-necrosis factor alpha (TNF-α) and interleukin 6 (IL-6) production has been demonstrated in individuals with sufficient magnesium intake. Furthermore, magnesium inhibits the activation of the Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF-kB) pathway, thereby reducing the production of inflammatory mediators. Magnesium supplementation has also been associated with a decrease in C-reactive protein (CRP) levels, a marker of systemic inflammatory status [4]. Thus, magnesium dampens the inflammatory cascade and reduces the risk of chronic inflammatory diseases.

Magnesium acts indirectly as an antioxidant by activating several antioxidant enzymes [5]. By promoting the activation of these enzymes, magnesium helps neutralize free radicals and reduce oxidative stress. Magnesium also plays a role in protecting cell membranes from oxidative damage. As a cofactor for many enzymes, it helps maintain redox balance and the stability of cell membranes. This is particularly important in endothelial cells, neurons, and heart cells, which are especially vulnerable to oxidative damage. Thus, through its activity on NF-kB pathway and pro-inflammatory cytokine production, and on antioxidant systems, magnesium prevents and modulates inflammatory and oxidative processes, thereby reducing the risk of diseases associated with inflammation and oxidative stress. Therefore, magnesium-based food supplements are used for a wide range of indications including fatigue, sarcopenia and bone function [6–8].

Although magnesium intake is relatively common in the diet, the bioavailability of this mineral is often low, and its deficiency is a widespread issue. Magnesium is absorbed in the small intestine, primarily in the duodenum and jejunum. About 30-50% of the magnesium ingested is absorbed, but this proportion can vary depending on dietary intake and the individual's physiological state [9]. Magnesium needs vary according to age, sex, and health conditions. Magnesium deficiency is common in modern populations due to unbalanced diets and increased physiological demands (stress, intense physical activity, etc.). Mg2+ deficiency may cause oxidative stress due to the increase in reactive oxygen species (ROS) that originate from mitochondrial dysfunction, activation of the renin-angiotensin-aldosterone system, and abnormal regulation of calcium homeostasis. In addition, Mg2+ deficiency also causes inflammation by increasing the production of proinflammatory molecules such as interleukin IL-1, IL-6, and TNF-α, which in turn can exacerbate the production of ROS. The combination of inflammation and oxidative stress induced by Mg2+ deficiency increases the risk of developing chronic diseases.

In this context, improving intestinal absorption and bioavailability of magnesium through the optimization of its formulations is a key area of research. Magnesium absorption in the intestinal tract is a complex process that can be influenced by several factors. When magnesium concentration in the intestinal lumen is high, absorption occurs passively via diffusion across the cell membranes according to a concentration gradient. When luminal concentrations are low, absorption takes place via an active process involving specific transporters in intestinal cells, such as Transient Receptor Potential Melastatin 6 (TRPM6) and TRPM7, two ion channels from the TRP family that facilitate the passage of magnesium through intestinal cells.

Strategies to optimize magnesium formulations aim to facilitate one or both of these absorption pathways by increasing magnesium solubility in the intestinal lumen or enhancing the expression of transporters responsible for its absorption. The bioavailability of magnesium in dietary supplements is largely determined by the chemical form of magnesium. Some forms are better absorbed than others due to their solubility, their ability to cross cell membranes, and their interaction with intestinal transporters [10, 11].

The magnesium oxide, although commonly used in dietary supplements due to its low cost and its high magnesium content, is generally poorly absorbed by the intestine, which limits its effectiveness. In contrast, other forms of magnesium show higher bioavailability, making them more promising choices in the context of magnesium deficiencies. The magnesium citrate is one of the most easily absorbed forms, due to its increased solubility in water. It dissociates easily in the intestine, facilitating its transport across cell membranes and entry into the bloodstream. Several studies have confirmed that magnesium citrate is more bioavailable than other forms of magnesium, such as magnesium oxide or magnesium sulfate [12, 13]. Additionally, this form is often well tolerated by the body, without significant side effects like gastrointestinal disturbances.

Chelated magnesium are new / more recent formulations of magnesium. Chelation involves the binding of magnesium ions to organic ligands—such as citrate, citrate malate, or bisglycinate—forming stable complexes that improve solubility, gastrointestinal absorption, and overall bioavailability. This structural modification also enhances tolerability by reducing gastrointestinal side effects commonly associated with inorganic magnesium salts, such as magnesium oxide.

The magnesium bisglycinate, a form of magnesium bound to glycine, is also well absorbed [14]. It is often recommended for individuals with digestive issues or heightened sensitivity to other forms of magnesium, as it has a lesser laxative effect. Research shows that this form has high bioavailability due to its ability to easily cross cell membranes and avoid forming insoluble complexes with food compounds that inhibit absorption. The magnesium citrate malate is a chelated magnesium salt formed by the combination of magnesium with citrate and malate anions. This complex is designed to enhance magnesium's solubility and gastrointestinal absorption, thereby improving its bioavailability compared to inorganic forms like magnesium oxide [13]. Citrate and malate, both intermediates in metabolic pathways, may offer additional benefits by supporting energy production and buffering acidity [15]. The supposedly improved absorption profile of magnesium citrate malate makes it a valuable source of magnesium for addressing deficiencies and supporting physiological functions, including muscle function, nerve transmission, and the modulation of inflammatory and oxidative stress responses.

This study aims to evaluate the absorption and biological activities of four distinct magnesium salts of marine origin: magnesium oxide, magnesium citrate, magnesium citrate malate and magnesium bisglycinate. Given the critical role of magnesium in numerous physiological processes, including anti-inflammatory and antioxidant pathways, improving its bioavailability is essential for maximizing biological effectiveness. Therefore, this study not only compares the bioavailability of these marine magnesium forms but also investigates their activity on inflammatory response and oxidative stress markers.

Materials and Methods

Cell Culture

Human colorectal adenocarcinoma cells (Caco-2) were cultured in Dulbecco's modified Eagle's medium (DMEM) (Gibco, Thermo Fisher Scientific) supplemented with 10% fetal bovine serum (Gibco, Thermo Fisher Scientific), 1% non-essential aminoacids (Gibco, Thermo Fisher Scientific) and 1% penicillin/streptomycin (Gibco, Thermo Fisher Scientific). Caco-2 cells were maintained and expanded at 37°C in a humidified atmosphere with 5% CO2. The medium was replaced every 2 days.

Caco-2 Permeability Assay

Caco-2 cells were harvested at 80% confluency using 0,05% trypsin and seeded at a density of 3.105 cells per 12 mm in the apical chamber of an insert (Corning). The DMEM medium was replaced in apical and basolateral compartments every 2 days. After 21 days of culture, the Caco-2 monolayer has become differentiated. Then, media were removed and Hanks' balanced salt solution (HBSS, Gibco, Invitrogen) was added in basolateral chambers. Apical chambers were treated with different magnesium salts (3mM) and apical and basolateral media were recovered after 1h and stored at -80°C.

Mice

C57BL/6 male mice aged 6-9 weeks were purchased from Janvier (France) and used for in vivo experiments, as previously described [16]. All mouse experiments were performed according to protocols approved by the institutional ethics committee (CEEA122) and the French Ministry of Higher Education, Research, and Innovation (ESRI) under permit number 5412201051917498658 in accordance with all animal experiments following the principles of animal care and use defined by the European legal and institutional guidelines (2010/63/UE). Animals per treatment group were housed in 425x266x185 mm cages (Tecniplast, 1291H Type III H, France) and given access to maintenance food (Global Diet, Harlan, France) and water ad libitum. The animals were not isolated in order to maintain the social behavior necessary for mice. The photoperiod was adjusted to 12 h light and 12 h dark and ambient temperature was maintained at 20°C +/- 1°C. Environmental enrichment included bedding and one hut.

Magnesium Bioavailability

The mice were randomized into four groups (five mice per group in total) and received one of the following marine magnesium salts by gavage (82 M) after fasting: marine magnesium oxide (Dr. Paul Lohmann GmbH & Co. KGaA), marine magnesium citrate (LoMarine® Citrate, Dr. Paul Lohmann GmbH & Co. KGaA), marine magnesium citrate malate (LoMarine® Citrate Malate, Dr. Paul Lohmann GmbH & Co. KGaA), and marine magnesium bisglycinate (LoMarine® Bisglycinate, Dr. Paul Lohmann GmbH & Co. KGaA). Each mouse was its own control at T= 0 h (0 % of Mg absorption). Marine Magnesium oxide was the Magnesium of reference. No exclusions were made. Changes in activity (isolation, breathing difficulties) were signs of pain signs that were *a priori* criteria for stopping the *in vivo* study.

Blood was collected at the tail vein with heparin capillaries (Sarstedt) at different times (0 min, 30 min, 60 min, 120 min, 240 min, 8 h and 24 h) to quantify magnesium levels.

Magnesium Quantification

Magnesium levels were measured with a sensitive kit, using the manufacturer's protocol (Magnesium assay kit, Abcam). The linear range of the assay is 2-15 nmoles with detection sensitivity ~40 μM.

Human PBMC isolation

Human peripheral blood mononuclear cells were isolated from the blood of healthy volunteers obtained from the Etablissement Français du Sang (EFS) Toulouse Purpan (France) using a density gradient centrifugation method on Ficoll (Cytiva). Monocytes were isolated from mononuclear cells by using AutoMACS (Pan monocyte isolation kit, Miltenyi Biotec).

Reverse Transcription and RT-qPCR

RNA from human monocytes treated with different Magnesium salts (3mM) for 24 h was isolated using the EZ-10 Spin Column Total RNA Minipreps Super Kit (Bio Basic) using the manufacturer's protocol. Synthesis of cDNA was performed according to the manufacturer's recommendations (Applied biosystem High-capacity cDNA Reverse Transcription kit). Quantitative reverse transcription polymerase chain reaction (qRT-PCR) was performed on a LightCycler 480 system using LightCycler SYBR Green I Master (Roche Diagnostics). The primers (Eurogentec) were designed with the software Primer 3. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA was used as the invariant control. Serially diluted samples of pooled cDNA were used as external standards in each run for the quantification. Primer sequences are listed in Table 1.

Table 1: Human primer sequences

Gene	5'-3' sequence
ARG1	Antisens CAC-TCT-ATG-TAT-GGG-GGC-TTASense CTT-GTT-TCG-GAC-TTG-CTC-GG
CAT	Antisens AGC-TTA-GCG-TTC-ATC-CGT-GTSense TCC-AAT-CAT-CCG-TCA-AAA-CA
COX2	Antisens CTG-CTC-AAC-AAC-GGA-ATT-TTSense GTG-CAC-TGT-GTT-TGG-AGT-GG
GAPDH	Antisens AGG-TCG-GAG-TCA-ACG-GAT-TTSense ATC-TCG-CTC-CTG-GAA-GAT-GG
HMOX1	Antisens ATG-ACA-CCA-AGG-ACC-AGA-GCSense GTG-TAA-GGA-CCC-ATC-GGA-GA
IL1B	Antisens CCA-GCT-ACG-AAT-CTC-CGA-CCSense TGG-ACC-AGA-CAT-CAC-CAA-GC
IL10	Antisens TGC-AAA-ACC-AAA-CCA-CAA-GASense TCT-CGG-AGA-TCT-CGA-AGC-AT
NOS2	Antisens GGC-AAG-CCC-AAG-GTC-TAT-GTSense CCT-CGA-CCT-GCT-CCT-CAT-TC
P47	Antisens CGG-TTG-GTG-GTT-CTG-TCA-GASense CAC-CTG-CAT-AGT-TGG-GCT-CA
TNFA	Antisens TCC-TTC-AGA-CAC-CCT-CAA-CCSense AGG-CCC-CAG-TTT-GAA-TTC-TT
TGFb1	Antisens CGC-GCA-TCC-TAG-ACC-CTT-TSense CTG-TGG-CAG-GTC-GGA-GAG-A
IL1RA	Antisens TCA-TTC-CAC-CTT-CCC-ATG-CCSense TAA-GTC-CTC-AGC-CTC-TCC-CC
NRF2	Antisens GCG-ACG-GAA-AGA-GTA-TGA-GCSense GTT-GGC-AGA-TCC-ACT-GGT-TT
GSR	Antisens TCA-GCT-CAC-CAC-AAC-CTC-TGSense GAG-ACC-AGC-CTG-ACC-AAC-AT
GP91	Antisens CCC-AAC-GAT-GCG-GAT-ATG-GASense TGA-GAG-GTT-GGT-GCG-GTT-TT

ROS Production

Human monocytes were treated with different marine magnesium (3mM) salts for 24 h and ROS production was measured by chemiluminescence in the presence of 5-amino-2,3-dihydro-1,4-phthalazinedione (luminol, Sigma) using a thermostatically (37°C) controlled luminometer (Envision, Perkin Elmer). The generation of chemiluminescence was monitored continuously for 1 h. Statistical analysis was performed using the area under the curve expressed in counts × seconds.

Statistical Analysis

Results are represented as mean +/- standard error of the mean (SEM) and statistical analyses were performed using two-way ANOVA followed by Tukey's multiple comparison test on GraphPad Prism (version 10) PRISM software.

Results

Intestinal absorption of marine magnesium oxide, marine magnesium citrate, marine magnesium bisglycinate, and marine magnesium citrate malate in Caco-2 permeability assay

Solubility and intestinal permeability are the most important factors determining oral absorption of mineral nutrients. Caco-2 (human colon adenocarcinoma cells) assay is a widely used model for evaluation of compound permeability properties. When grown to confluence and allowed to differentiate the cells form a monolayer resembling luminal epithelium of human intestine by structure and properties. Caco-2 cells have a variety of active transporters, which are relevant to the absorption process in the gastro-intestinal tract. Therefore, Caco-2 method is more suitable for the prediction of *in vivo* compound efflux. To evaluate the intestinal permeability of various salts of marine magnesium, magnesium oxide, magnesium citrate, magnesium citrate malate, and magnesium bisglycinate have been added into the apical compartment of the trans-well insert, and the appearance of these magnesium on the basolateral side after incubation time of 1 h have been quantified (Figure 1). The magnesium concentration attributable to marine magnesium oxide in the basolateral compartment is lower than in the other marine salts, indicating that it has the lowest absorption rate among all the tested forms (Figure 1A-B). In contrast, marine magnesium citrate exhibits the highest concentration in the basolateral compartment, with an absorption rate approximately three times higher than marine magnesium oxide. For the marine magnesium bisglycinate the absorption is higher than marine magnesium oxide but remains lower than marine magnesium citrate. The marine magnesium bisglycinate is approximately 2 times better absorbed than marine magnesium oxide. Similarly, marine magnesium citrate malate also demonstrates significantly higher absorption than marine magnesium oxide, although it is slightly less than marine magnesium citrate, achieving approximately twice the absorption of marine magnesium oxide. Thus, marine magnesium oxide exhibits poor absorption, making it the least efficient form of marine magnesium for bioavailability in this *in vitro* Caco-2 model. Organic marine magnesium compounds (citrate, bisglycinate, and citrate malate) are significantly more effective in terms of absorption, with citrate leading the group.

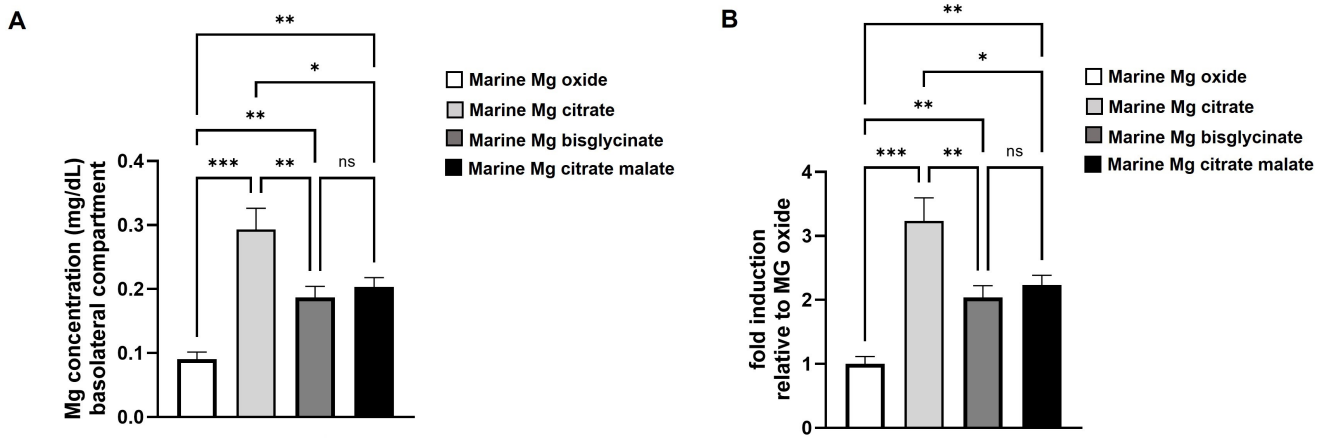

Figure 1: Intestinal absorption of marine magnesium oxide, marine magnesium citrate, marine magnesium bisglycinate, and marine magnesium citrate malate. (A-B) Magnesium quantification in mg/dL (A) or in fold induction compared to Mg oxide (= 1) (B) in the basolateral compartment after incubation for 1 hour of Caco-2 cell line with marine Mg oxide (white), marine Mg citrate (light grey), marine Mg bisglycinate (dark grey) or marine Mg citrate malate (black). Statistical significance: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns : not significant.

In vivo bioavailability of marine magnesium salts

The profile of marine magnesium oxide adsorption shows a rapid increase in serum magnesium levels (15% absorption) from 30 min following the acute ingestion, reaching a plateau of absorption (19%) between 2 and 4 hours, followed by a progressive decrease to 24h post ingestion (Figure 2A-E). The marine magnesium citrate is more bioavailable than marine magnesium oxide. Indeed, maximal serum magnesium levels are higher than with marine magnesium oxide (25% absorption) (Figure 2A-B). The absorption profile of marine magnesium citrate shows a progressive and continuous increase in magnesium level absorbed up to 24 hours after acute ingestion, indicating a more efficient and prolonged absorption. For marine magnesium bisglycinate, the absorption profile shows a high peak at 4 hours following acute ingestion (27% absorption), suggesting efficient absorption followed by rapid elimination (Figure 2C). The marine magnesium citrate malate presents the highest absorption at 30 min after acute ingestion (19% absorption) (Figure 2D-E). There is a progressive increase with a peak at 4 hours post ingestion, with a maximal serum magnesium increase at 23%.

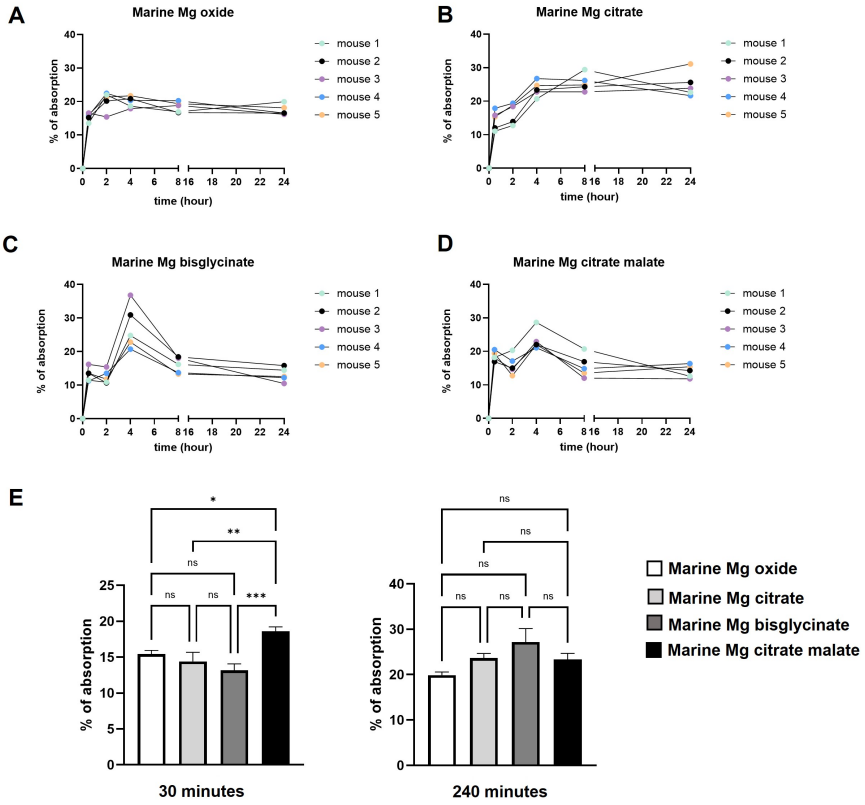

Figure 2: *In vivo* bioavailability of marine magnesium forms (oxide, citrate, bisglycinate, and citrate malate) in murine serum. (A-D) Percentage of magnesium absorption in serum of orally-treated mice at different time points with marine Mg oxide (A), marine Mg citrate (B), marine Mg bisglycinate (C) or marine Mg citrate malate (D). (E) Percentage of magnesium absorption at 30 minutes (left panel) and 240 minutes (right panel) in serum of orally-treated mice with marine Mg oxide, marine Mg citrate, marine Mg bisglycinate or marine Mg citrate malate. Statistical significance: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns: not significant.

The findings demonstrate that marine magnesium citrate, bisglycinate, and citrate malate offer superior absorption compared to marine magnesium oxide. Marine magnesium bisglycinate exhibits the highest peak absorption, whereas marine magnesium citrate and marine magnesium citrate malate display high and stable absorption, with the advantage of citrate-malate exhibiting rapid absorption. This suggests that these three marine organic forms may be preferable choices for maintaining stable magnesium levels and optimizing bioavailability, while marine magnesium oxide appears to be the least efficient option.

Impact of marine magnesium salts on the inflammatory response and oxidative stress

To explore the anti-inflammatory activities of different salts of marine magnesium, we evaluated the gene expression of pro- and anti-inflammatory cytokines and cyclooxygenase 2 (COX-2), a key mediator of inflammatory pathways, in treated human monocytes, the main cells involved in the regulation of inflammatory response (Figure 3A). All marine magnesium forms significantly decrease the expression of mRNAs encoding pro-inflammatory cytokines (TNF-α, IL-1β). Although marine magnesium oxide decreases the expression of pro-inflammatory cytokines, it also downregulates the expression of transforming growth factor beta (TGF-β) and IL-10 anti-inflammatory factors. Interestingly, the inhibition of pro-inflammatory cytokines observed with the marine organic magnesium compounds (citrate, bisglycinate, and citrate malate) is mirrored by a significant increase of TGF-β, IL-10 and IL-1ra anti-inflammatory factors, with a stronger effect of marine magnesium bisglycinate, and marine magnesium citrate malate. In line, the mRNA level of COX-2 in monocytes is strongly reduced with all forms of marine magnesium with a stronger effect of marine organic magnesium compounds (citrate, bisglycinate, and citrate malate). The transcriptional master regulator of cellular responses against oxidative stress, known as nuclear factor erythroid 2-related factor 2 (Nrf2), is among the key factors that regulate the redox balance. Indeed, Nrf2 controls the expression of multiple anti-oxidant and phase II enzyme genes [17]. In this context, we assess the expression of Nrf2 and its target genes such as heme oxygenase (HMOX1), Catalase (CAT) and Glutathione reductase (GSR) antioxidant enzymes. The expression of both Nrf2 and its target genes are upregulated by marine organic magnesium forms (citrate, bisglycinate, and citrate malate) with a stronger effect of marine bisglycinate, and citrate malate forms. Marine magnesium oxide up-regulates only HMOX1, while it inhibits catalase and GSR expression, demonstrating that the antioxidant effect of marine magnesium oxide is weaker than the other salts.

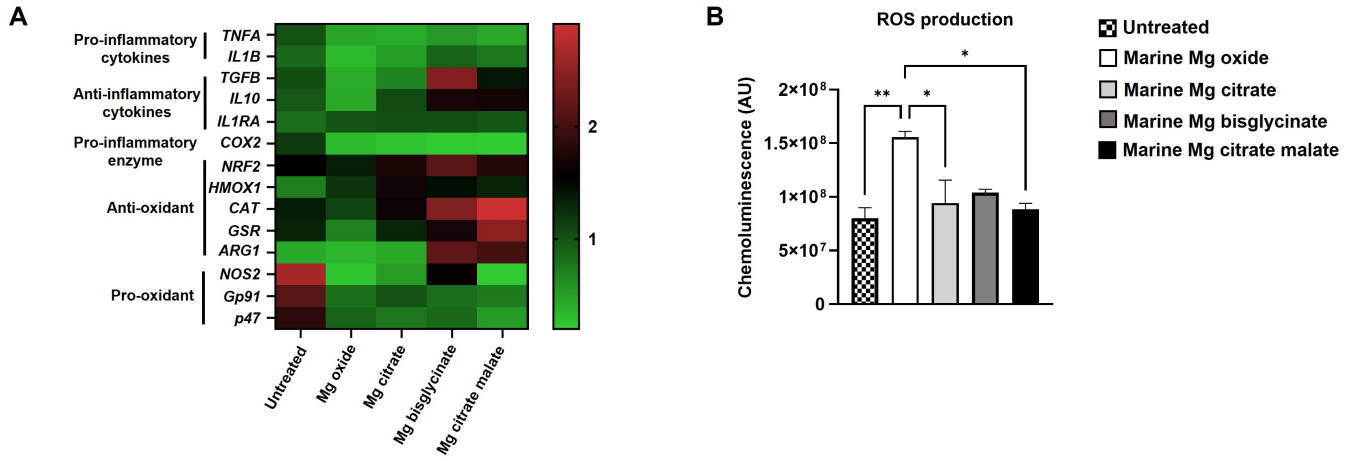

Figure 3: Impact of marine magnesium salts (oxide, citrate, bisglycinate, and citrate malate) on the inflammatory response and oxidative stress. (A) Heatmap showing mRNAs expression of pro- and anti-inflammatory cytokines, arachidonic acid metabolic enzymes, anti-oxidant genes and NADPH oxidase subunits by human monocytes treated or not with the different marine Mg salts (below 1 = down-regulation, above 1 = up-regulation). (B) ROS production by human monocytes treated or not with the different marine Mg salts. Statistical significance: * $p < 0.05$, ** $p < 0.01$. Results are representative of 3 biological replicates, each with 3-4 technical replicates.

Moreover, all forms of marine magnesium strongly reduce the expression of NOS 2 (inducible nitric oxide synthase). Only marine magnesium citrate, b marine magnesium bisglycinate, and marine magnesium citrate malate increase the expression of Arginase-1 (ARG1), an enzyme strongly involved in the inhibition of NO production. In contrast to the induction of Arginase-1 expression by marine magnesium citrate, marine magnesium bisglycinate, and marine magnesium citrate malate, marine magnesium oxide decreases Arginase-1 expression. Thus, treatment of human monocytes with marine organic magnesium salts (citrate, bisglycinate, and citrate malate) orients the balance between NOS2 and Arginase-1 toward Arginase-1 expression, demonstrating that these forms amplify the antioxidant activity of human monocytes. In line, the mRNA level of Gp91

and p47phox, cytosolic subunits of the Nicotinamide Adenine Dinucleotide Phosphate Hydrogen (NADPH) oxidase complex whose activation is essential to ROS release, are significantly decreased in human monocytes treated by all the marine magnesium salts. Interestingly, only the marine magnesium oxide triggers the production of ROS by human monocytes (Figure 3B). The treatments with marine magnesium citrate, bisglycinate, and citrate malate salts have no impact on the ROS release of human monocytes.

Altogether these results demonstrate that marine organic magnesium compounds (citrate, bisglycinate, and citrate malate) exhibit much more robust anti-inflammatory and antioxidant activities than marine magnesium oxide. Interestingly, marine magnesium bisglycinate and marine magnesium citrate malate have stronger anti-inflammatory and antioxidant activities than marine magnesium citrate form.

Discussion

The findings highlighted from this study provide valuable insights into the intestinal absorption, bioavailability, anti-inflammatory and antioxidant properties of various magnesium salts of marine origin: magnesium oxide, citrate, bisglycinate, and citrate malate. Our investigation reveals notable differences in their bioavailability, absorption rates, and impact on inflammatory responses and oxidative stress, which are key factors in determining the efficacy of magnesium supplementation. The results of the Caco-2 permeability assay indicate that marine magnesium oxide exhibits the lowest intestinal absorption rate among the marine salts tested. Specifically, the concentration of magnesium oxide marine in the basolateral compartment was significantly lower compared to the other forms, suggesting that it is the least bioavailable. This is consistent with previous studies indicating that magnesium oxide is poorly absorbed due to its low solubility in the gastrointestinal tract.

In contrast, marine magnesium citrate demonstrated the highest absorption rate, with an absorption rate approximately three times higher than marine magnesium oxide. This enhanced absorption can be attributed to the high solubility and favorable intestinal permeability of magnesium citrate, making it the most efficient form for bioavailability in this *in vitro* model. Similarly, marine magnesium bisglycinate and citrate malate exhibited significantly higher absorption than marine magnesium oxide approximately twice as bioavailable. These results are consistent with existing data indicating that organic forms of magnesium are generally better absorbed than inorganic forms.

These findings are corroborated by the *in vivo* bioavailability study in mice, which shows that marine magnesium citrate and marine magnesium citrate malate achieve high serum magnesium levels, with a steady increase in magnesium levels over 24 hours for the marine magnesium citrate and a rapid and efficient absorption for marine citrate malate. This suggests that marine magnesium citrate and marine magnesium citrate malate are not only more bioavailable than marine magnesium oxide but also offer prolonged absorption for the marine magnesium citrate, which could be beneficial for sustained magnesium supplementation, and a rapid absorption for the citrate malate, better suited to situations requiring more rapid intake. Marine magnesium bisglycinate, while showing a higher peak absorption, has a more rapid decline, making it better suited for situations requiring acute absorption. Marine magnesium oxide, on the other hand, exhibited limited absorption with a relatively low maximum serum level (19% absorption), confirming its poor bioavailability *in vivo*.

The different organic forms of magnesium exhibit distinct metabolic fates following absorption. Indeed, citrate and malate can contribute directly to the Krebs cycle, thereby playing an integral role in cellular energy metabolism [18]. In contrast, bisglycinate can dissociate into glycine, which enters the amino acid pool and is primarily utilized in protein synthesis and other amino acid-dependent pathways [19]. These divergent metabolic pathways suggest that the physiological effects of magnesium supplementation may vary depending on the compound involved. Therefore, further studies are needed to comprehensively evaluate the post-absorptive fate of these organic magnesium complexes, as understanding their specific metabolic trajectories could op-

timize supplementation strategies.

The anti-inflammatory effects of marine magnesium forms were assessed by evaluating cytokine gene expression and COX-2 levels in human monocytes. All marine magnesium forms were found to significantly reduce the expression of pro-inflammatory cytokines (TNF-α, IL-1β), which is consistent with previous reports highlighting the anti-inflammatory properties of magnesium. However, the marine organic magnesium compounds (citrate, bisglycinate, and citrate malate) showed a more pronounced effect by increasing the expression of anti-inflammatory factors such as TGF-β, IL-10, and IL-1ra. These effects were notably stronger in marine bisglycinate and citrate malate salts, suggesting that these forms may offer more potent anti-inflammatory benefits compared to marine magnesium oxide.

In terms of oxidative stress, the transcription factor Nrf2, a key regulator of cellular antioxidant responses, was significantly upregulated in human monocytes treated with marine magnesium citrate, marine magnesium bisglycinate, and marine magnesium citrate malate. This upregulation led to increased expression of antioxidant enzymes such as catalase and glutathione reductase, which are essential for protecting cells from oxidative damage. In contrast, marine magnesium oxide only upregulated the expression of HMOX1 and inhibited the expression of catalase and glutathione reductase, indicating a weaker antioxidant effect. Moreover, marine magnesium oxide was the only form that triggered the production of ROS, which suggests that it may have pro-oxidative effects, potentially negating some of its benefits.

The differential effects on NOS2 and Arginase-1 expression also reflect the varying capacities of these marine magnesium forms to modulate oxidative stress. While all forms of marine magnesium reduced NOS2 expression, only citrate, bisglycinate, and citrate malate increased the expression of Arginase-1, an enzyme involved in inhibiting nitric oxide production and reducing inflammation. This shift in the Nos2/Arginase-1 balance toward Arginase-1 expression in marine organic magnesium forms further supports their superior antioxidant and anti-inflammatory effects. Once absorbed into the bloodstream, all marine magnesium forms release magnesium in its free form rather than remaining complexed. Therefore, the antioxidant and anti-inflammatory properties of the native forms may be particularly beneficial at the digestive level, potentially protecting the intestine from inflammatory and oxidative damage.

Several limitations of the *in vivo* study should be highlighted. First, marine magnesium supplementation in mice was administered on an empty stomach. As a result, the gastric pH was very low, potentially favoring the absorption of marine magnesium oxide, which is soluble only in highly acidic conditions. In contrast, marine magnesium citrate, marine magnesium citrate malate, and marine magnesium bisglycinate remain soluble even at neutral pH. Repeating the study with magnesium supplementation taken alongside a meal would provide valuable insights. Second, conducting a study with prolonged magnesium supplementation over several weeks would be beneficial to assess long-term bioavailability and effects.

Conclusion

These results demonstrate that marine organic magnesium compounds, particularly magnesium citrate, bisglycinate, and citrate malate, are significantly more effective in terms of both intestinal absorption and bioavailability compared to marine magnesium oxide. Additionally, these marine organic forms exhibit more robust anti-inflammatory and antioxidant activities, with bisglycinate and citrate malate showing stronger effects than magnesium citrate. Marine magnesium oxide, with its limited absorption and weaker anti-inflammatory and antioxidant properties, appears to be the least effective form of marine magnesium supplementation in the studied parameters.

In clinical applications, marine magnesium citrate, marine magnesium bisglycinate, and marine magnesium citrate malate may be the preferred options for individuals seeking efficient and prolonged absorption, and marine magnesium citrate malate could be also more suitable for cases requiring rapid magnesium absorption. Given the superior bioavailability and broader

therapeutic benefits of the marine organic magnesium compounds, their use could be recommended for patients aiming to address both magnesium deficiency and inflammation-related conditions.

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Conflicts of Interest

C.G. and H. B. are employees of Dr. Paul Lohmann GmbH & Co. KGaA and C.B.B. is employed by the company C2B Conseil. The authors declare that this study received funding from Dr. Paul Lohmann GmbH & Co. KGaA. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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