Effects of Magnesium Forms on the Magnesium Balance and Jejunal Transporters in Healthy Rats

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ABSTRACT: Magnesium (Mg) is a mineral necessary for many biological activities in mammals. Here, we compared the effect of two Mg compounds [Mg picolinate (MgPic) to Mg oxide (MgO)] on Mg bioavailability and intestinal Mg and calcium transporter protein levels. Three groups of 21 male Wistar-Albino rats were randomly allocated and fed a standard diet (control) or a 500 mg/kg Mg-supplemented (MgPic or MgO) diet for 8 weeks. The serum and liver Mg levels, Mg absorptivity, and retentivity were augmented in the MgPic group compared with the MgO group ($P<0.05$). Only MgPic supplementation elevated the expression of the genes encoding CLDN2, CLDN15, CNNM4, NCX1, PMCA1b, NCX2, and Calbindin-D9k in the jejunum by 1.59, 1.58, 1.70, 1.82, 2.02, 2.03, and 2.31 fold, respectively ($P<0.05$). Compared to the MgO-fed rats, MgPic rats had higher expression of the genes encoding NCX1, NCX2, PMCA1b, and Calbindin-D9k in the jejunum by 1.43, 1.72, 1.54, and 1.69 fold, respectively ($P<0.01$). These results suggest that MgPic increases Mg absorptivity and retentivity more than Mg bioavailability. In addition, MgPic can improve the paracellular and transcellular cationic mineral transport process. Thus, Mg deficiency disorders might be alleviated by MgPic more effectively than MgO.

Keywords: bioavailability, biological transport, dietary supplements, magnesium oxide, picolinic acid

INTRODUCTION

Magnesium (Mg) is essential for the optimal functioning and regulation of various metabolic processes in the body, playing a central role in maintaining the overall physiological balance and health. It acts as an important cofactor for more than 600 enzymes, playing an integral role in biochemical reactions critical to cellular functions, including energy production, genetic processes, and physiological regulation.

Inadequate Mg impairs these processes, making it important to overall health and well-being (Fiorentini et al., 2021).

Mg homeostasis is primarily maintained through the intricate cellular and molecular interactions between intestinal absorption and renal transport mechanisms. This delicate balance ensures the body can effectively regulate Mg levels, which are critical for numerous physiological functions (Houillier, 2014; de Baaij et al., 2015; Houillier et al., 2023). The entire intestinal tract contributes to Mg absorption, with approximately 89% of Mg absorbed by the small intestines and about 11% by the colon (Workinger et al., 2018; Raya-Sandino et al., 2023). When the intestinal Mg ion concentration is high, passive intestinal transport (paracellular diffusion) provides 80% of Mg absorption in the small intestines. Conversely, the transcellular transport system becomes dominant at low luminal Mg concentrations for maintaining Mg homeostasis (Kiela and Ghishan, 2018).

Different Mg carriers regulate these transport systems. For example, the paracellular Mg transport system is regulated by an integral membrane protein, claudin, which controls tight junction permeability (Günzel and Yu, 2013). Sodium (Na)-calcium (Ca) exchanger (NCX) proteins are responsible for the bidirectional exchange of Ca/Na ions across the cell membrane. In physiological conditions, Mg ions compete with Ca ions for binding to NCX1 and can reduce the NCX1's affinity for Ca ions (Levitsky and Takahashi, 2013). As a result, NCX1 is also involved in regulating the extrusion of Mg ions from the cell; in other words, the presence of Mg can influence how effectively NCX1 transports Ca and Na, in turn affecting Mg removal from the cell (Tashiro et al., 2000).

Meanwhile, plasma membrane Ca-ATPase 1b (PMCA1b) primarily governs the extrusion of Ca ions from enterocytes, playing a crucial role in maintaining Ca homeostasis in these cells. In contrast, NCX1 contributes to a smaller extent, accounting for only approximately 20%

of Ca ion extrusion. Thus, while PMCA1b is the predominant transporter for removing excess Ca from enterocytes, NCX1 plays a supplementary role in the process (Diaz de Barboza et al., 2015; Goff, 2018).

In addition, cyclin-M4 (CMNN4), a key mediator of transcellular Mg ions transport, is predominantly localized to the basolateral membrane of enterocytes, which are essential for Mg transport across the cell membrane (Yamazaki et al., 2013). Calbindin-D9k contributes to the intracellular transport of Ca and Mg, promoting their effective intracellular mobility and controlling their concentrations in different cellular compartments. Calbindin-D9k helps regulate intracellular Ca and Mg concentration by binding and transporting them within the cell, contributing to their overall homeostasis and functional availability (Andersson et al., 1997).

Also, inorganic minerals compete for the same transport carriers during their transfer from the intestinal lumen into enterocytes, reducing each other's uptake, as the availability of binding sites on the carriers is limited (Vieira, 2008). In contrast, organic mineral chelates feature an organic ligand that binds to the mineral, effectively masking the mineral's positive charge, reducing electrostatic interactions with the intestinal mucus layer, facilitating a faster passage through the barrier, and potentially enhancing mineral absorption (Power, 2006). Chelated metal ions do not compete with other minerals for the binding sites on transporters (Power, 2006) and are passed through enterocytes quickly using their chelators as transporters (Vinus, 2017).

Many inorganic and organic Mg salts are commercially available as nutrition supplements. These substances include Mg salts in two different forms: inorganic salts [such as Mg oxide (MgO) and sulfate] and organic salts (such as Mg citrate and Mg glycinate). While organic salts are created by bonding Mg with an organic compound or an amino acid, inorganic salts are usually obtained from mineral sources. This process can affect salt absorption and bioavailability in the body. Each type of Mg salts has distinct properties that affect its absorption, bioavailability, and suitability for different nutritional needs (Calbet et al., 2011). For example, inorganic Mg salts have high Mg content but low solubility and, thus, reduced bioavailability. In contrast, organic Mg forms are more soluble and have higher bioavailability (Blancquaert et al., 2019). In addition, a novel organic form of Mg picolinate (MgPic), which contains Mg and picolinic acid (PA), has a higher Mg solubility in the body pH range than many other Mg forms. Therefore, MgPic likely exhibits greater bioavailability than other Mg forms (Nelson and Komorowski, 2018; Orhan et al., 2022). However, the effect of MgPic on intestinal Mg and Ca transporters at the molecular level has not been studied.

Here, we aimed to investigate the impacts of MgPic on several key parameters, including serum and liver Mg levels, as well as Mg absorption and retention. Compared to other forms, such as MgO, MgPic has been shown to have higher absorption rates, making it a more effective option for studies where optimal Mg uptake is crucial. Additionally, we aimed to examine the impact of MgPic on the intestinal expression of various proteins, namely CLDN2, CLDN12, CLDN15, NCX1, NCX2, PMCA1b, CNNM4, and Calbindin-D9k.

MATERIALS AND METHODS

Animal and feeding

Twenty-one male Wistar-Albino rats, all aged eight weeks and weighing 170±20 g, were housed under carefully regulated conditions. They were provided with unrestricted access to water and food for their nutritional needs. The living environment was maintained at 22°C±2°C, with a relative humidity of 55%±5%, and a consistent 12-h light-dark cycle to create an optimal setting. The rats were fed with the standard diet (Table 1) or a diet supplemented with MgO (Hard Eight Nutrition LLC) or MgPic (Nutrition 21). The dose of Mg (500 mg/kg diet) was determined according to Bertinato et al. (2014). Body weight was measured weekly. All protocols were approved by the Animal Ethics Committee at Firat University. The study was approved by the Animal Ethics Committee of Firat University (156-2017/86) and performed following the internationally accepted standard

Table 1. Composition of the basal diets fed to rats[1]

	Value (%)
Starch	57.95
Casein	20.00
Soybean oil	7.00
Sucrose	5.00
Cellulose	5.00
Mineral premix[2]	3.00
Vitamin premix[3]	1.50
L-Cysteine	0.30
Choline bitartrate	0.25

[1]The diet was formulated to include 478.8±45.2 mg of magnesium per kilogram.
[2]The mineral premix for the formulation contained the following components per kilogram of the mixture: calcium carbonate anhydrous 357 g, potassium phosphate monobasic 196 g, sodium chloride 74 g, potassium sulfate 46.6 g, potassium citrate monohydrate 70.78 g, ferric citrate 6.06 g, zinc carbonate 1.65 g, manganous carbonate 0.63 g, cupric carbonate 0.30 g, potassium iodate 0.01 g, anhydrous sodium selenate 0.01025 g.
[3]Vitamin premix (mixture, g/kg): niacin 3, Ca-pantothenate 1.6, pyridoxine-HCl 0.7, thiamine HCl 0.6, riboflavin 0.6, folic acid 0.2, D-biotin 0.02, vitamin B12 (0.1% cyanocobalamin in mannitol) 2.5, vitamin E (all-rac-α-tocopheryl acetate, 500 IU/g) 15, vitamin A (all-trans-retinyl palmitate, 500,000 IU/g) 0.80, vitamin D3 (cholecalciferol, 400,000 IU/g) 0.25, vitamin K (phylloquinone) 0.075.

ethical guidelines for laboratory animal use and care as described in the European Community guidelines, EEC Directive 2010/63/EU, of September 22, 2010.

As a study design, the rats were randomized into three groups (with seven in each): 1) Control group (standard diet, Table 1); 2) MgO group (a diet supplemented with MgO, with 500 mg of Mg per kg); 3) MgPic group (a diet supplemented with MgPic, with 500 mg of Mg per kg). The experiment went on for eight weeks and was concluded by euthanizing the rats. Afterward, samples of the rat jejunum and serum were collected and preserved at $-80°C$ until they were analyzed.

Magnesium absorption and retention

First, the Mg balance was examined during the final five days of the experiment. Each rat was individually placed in a designated metabolic cage, allowing for precise monitoring of metabolic processes and ensuring controlled environmental conditions. They were fed the number of the identical experimental diets at 8:00 every day, and daily feed intake was measured. Samples of urine and feces for each group were taken at the end of the day and pooled for 5 days. Pooled feces samples were dried at 65°C, whereas urine samples were stored at $-20°C$.

Intestinal absorption of Mg was calculated as Mg intake (mg/d)$-$Mg ions excreted in the feces (mg/d), and Mg retention was calculated as Mg absorption (mg/d)$-$ Mg excreted in the urine (mg/d). The percentage of Mg absorptivity was calculated as Mg absorption (mg/d)/Mg intake (mg/d)$×100\%$, and the percentage of Mg retention was calculated as Mg retention (mg/d)/Mg intake (mg/d)$×100\%$.

Biochemical and elemental analysis

Serum was obtained by centrifuging rat blood samples at 3,000 g for 10 min. After centrifugation, the clear supernatant was collected. Then, the serum levels of various biomarkers were measured using rat-specific kits and an autoanalyzer (Samsung Labgeo PT10V, Samsung Electronics). The biomarkers included glucose and insulin for monitoring blood sugar regulation, cholesterol and triglycerides for assessing lipid metabolism, and free fatty acids (FFAs) for examining fat utilization. Additionally, creatinine levels were measured to evaluate kidney function, and alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were analyzed to gauge liver health. Also, blood urea nitrogen (BUN) was assessed to investigate metabolism and renal function. Together, these measurements offered a comprehensive view of the metabolic, liver, and overall biochemical health of the rats.

Namely, 0.3 g of liver, feed, and feces samples and 0.5 mL of serum and urine were weighed, put in Teflon tubes, and digested with 65% HNO_3 (Merck) in a microwave digestion device (Speedwave^{TM} MWS-2, BergHOFF)

using various digestion programs to determine Mg content. For the feed, the system was set to run at 80% power and 145°C for 10 min. Then, it was run at 80% power and 160°C for 10 min. Next, it was run at 80% power and 90°C for 20 min. For the liver, excrement, serum, and urine samples, the system was run at 80% power and 130°C at 8 min, 80% power and 155°C for 5 min, and 80% power and 170°C for 12 min.

In addition, a flame atomic absorption spectrophotometer (Perkin-Elmer, Analyst 800) was used to measure Mg levels in the digested solutions after they had been brought up to a final volume of 100 mL with deionized water.

Isolation of total RNA and real-time PCR analysis

First, a jejunal segment from the small intestine was preserved in RNA and purified using a commercial RNA extraction kit (RNeasy 96 Universal kit, QIAGEN). After 40 mg of a tissue sample was weighed, it was homogenized using a homogenizer (Tissue Lyser LT, QIAGEN) with 600 μL of 1:100 beta-mercaptoethanol in RLT buffer. Following a three-minute centrifugation, the supernatant was taken to combine with 70% ethanol.

Also, total RNA was extracted using the RNeasy Mini Kit (QIAGEN) method and automated by the QIAcube system (QIAcube, QIAGEN) and stored in 1.5-mL vials at $-80°C$. Then, the absorbance ratio (A260/A280) of the RNA samples was determined using a NanoDrop spectrophotometer (MaestroGen); a ratio of approximately 2 indicated an adequate RNA concentration.

After total RNA extraction, the target genes were amplified using specific primers. The sequences of the primers for various genes were as follows: for CLDN2 (Gene ID: 300920), 5'-GCTGCTGAGGGTAGAATGA-3' (forward primer) and 5'-GCTCGCTTGATAAGTGTCC-3' (reverse primer); for CLDN12 (Gene ID: 500000), 5'-AACTGGCC AAGTGTCTGGTC-3' (forward) and 5'-AGACCCCCTGAG CTAGCAAT-3' (reverse); for CLDN15 (Gene ID: 304388), 5'-CGCTTGGCAGGGTGTTTGGTCATA-3' (forward and reverse); for NCX1 (Gene ID: 29715), 5'-TGTTCTTTGT GACAGCAGCC-3' (forward) and 5'-AAGGAGACAGAC CAGCTTCC-3' (reverse); for NCX2 (Gene ID: 140447), 5'-AGCTATGAAGCCGTTCTCTGCTC-3' (forward) and 5'-GGATGTTGCTCAAAGGCACAGTAC-3' (reverse); for PMCA1b (Gene ID: 29598), 5'-CGCCATCTTCTGCACA ATT-3' (forward) and 5'-CAGCCATTGTTCTATTGAAA GTTC-3' (reverse); for CNNM4 (Gene ID: 363216), 5'-GAGGAGCATGGGAGGTTTCT-3' (forward) and 5'-CGA GCGTACCTTCTCTCCTT-3' (reverse); for Calbindin-D9k (Gene ID: 24249), 5'-ACCTCACCTGTTCCTGTCTG-3' (forward) and 5'-CAGTCAGGTTAGGGGCTAGG-3' (reverse); for the housekeeping gene GAPDH (Gene ID: 24383), 5'-GCCAGCCTCGTCTCATAGACA-3' (forward) and 5'-AGAGAAGGCAGCCCTGGTAAC-3' (reverse). Each

of these primers underwent a homology search to confirm their specificity for their target mRNA transcripts. GAPDH, a commonly used housekeeping gene, served as a control to ensure accurate normalization of gene expression data. The primers were sourced from QIAGEN.

The National Center for Biotechnology Information database (USA), specifically GenBank®, was utilized for gene information and sequence data. The total RNA expression levels were quantified using an RT-PCR Detection System (QIAGEN). A first-strand cDNA synthesis kit (QIAGEN) was used to convert total RNA into complementary DNA (cDNA). Next, 1 μL of the synthesized cDNA was combined with 5 μL of 2X SYBR Green Master Mix (FastStart Universal SYBR Green Master Mix, QIAGEN) and the designated primer pairs. The total volume of this reaction mixture was brought up to 20 μL. Then, this preparation underwent 40 heat cycles of qRT-PCR (Rotor-Gene Q, QIAGEN); green fluorescence was measured at the conclusion of each extension step. Initial denaturation was conducted at 95°C for 10 min, followed by denaturation at 95°C for 15 s, annealing at 65°C for 30 s, and extension at 72°C for 15 s. The relative expression levels of each gene were calculated by normalizing to GAPDH and adjusting for efficiency using tools from the QIAGEN GeneGlobe Data Analysis Center. Fold changes in gene expression were calculated using the $2^{-\Delta\Delta CT}$ method, presenting the results relative to the control group. Differential gene expression was assessed through pairwise comparisons, applying a fold-change threshold of two and a significance level of 0.05 (P<0.05) to identify statistically significant differences.

Statistical analyses

An appropriate sample size (n=21) was ensured for the study using G*Power (Version 3.1.9.4), with parameters at an alpha error of 0.05, an effect size of 0.85, and 90% power. Multiple comparisons were conducted by initially using a one-way ANOVA to determine the overall differences among the groups and then utilizing post hoc Tukey's HSD tests to identify specific pairwise differences and determine statistical significance. A significance threshold was set at a P-value of 0.05. Results were presented as mean±standard deviation. All statistical analyses were performed using SPSS (version 22.0, IBM Corp.) to ensure robust and accurate data interpretation.

RESULTS

Both MgO and MgPic had no significant effect on the feed intake or body weight for the groups (P>0.05; Fig. 1). Similarly, they both did not significantly affect the serum levels of glucose, insulin, cholesterol, triglycerides, FFA, ALT, AST, creatinine, or BUN (P>0.05; Fig. 1).

These findings suggest that MgO and MgPic had no meaningful impact on these physiological and biochemical measures.

Meanwhile, Mg-supplemented rats had significantly higher serum and liver Mg levels than control rats (Table 2). Serum Mg increased in rats supplemented with MgO and MgPic in comparison to the control group (P<0.01 and P<0.001, respectively). In addition, liver Mg levels increased in the MgO and MgPic groups compared with the control group (P<0.05 and P<0.001, respectively). Also, the MgPic group had higher serum and liver Mg levels than the MgO group (P<0.01 for both). In contrast, MgO supplementation did not influence the feces and urine Mg levels compared to the control group (P>0.05). Meanwhile, the MgPic group had reduced feces and urine Mg levels compared to the MgO and control groups (P<0.01 for both). Mg absorption and retention were significantly higher in the MgO and MgPic groups than in the control group (P<0.001). In addition, there were no significant differences in the Mg absorption and retention between the MgO and MgPic groups (P>0.05).

The increase in Mg absorption and retention suggested an increase in the expression of the genes encoding key Mg transporter proteins, particularly NCX1 and PMCA1b. These proteins are increased by MgPic supplementation (Fig. 2), indicating improved Mg transport and cellular uptake, which is a crucial biological response identified in this study. This finding indicates that both forms of Mg had similar effects on Mg absorption and retention. In contrast, Mg absorptivity was 83.77%, 91.65%, and 94.05% in the control, MgO, and MgPic groups, respectively (Table 2). In addition, Mg retentivity was 70.58%, 84.92%, and 88.01% in the control, MgO, and MgPic groups, respectively. Therefore, the MgPic group had significantly higher Mg absorptivity than the control and MgO groups (P<0.001).

The effects of MgO and MgPic on the expression of jejunal Mg transporter genes were examined (Fig. 2). Compared to the control group, only MgPic elevated the expression of the genes encoding CLDN2, CLDN15, and CNNM4 by 1.59, 1.58, and 1.70 folds, respectively (P<0.05). In addition, MgPic increased the expression of the genes encoding NCX1 and PMCA1b by 1.82 and 2.02 folds, respectively (P<0.001). MgPic also enhanced the expression of the genes encoding NCX2 and Calbindin-D9k by 2.03 and 2.31 folds, respectively (P<0.01). Besides, compared to the MgO group, the MgPic group had higher expression of the genes encoding NCX1, NCX2, PMCA1b, and Calbindin-D9k by 1.43, 1.72, 1.54, and 1.69 folds, respectively (P<0.01). Moreover, both Mg forms did not significantly increase the expression of the jejunal CLDN12 gene (P>0.05).
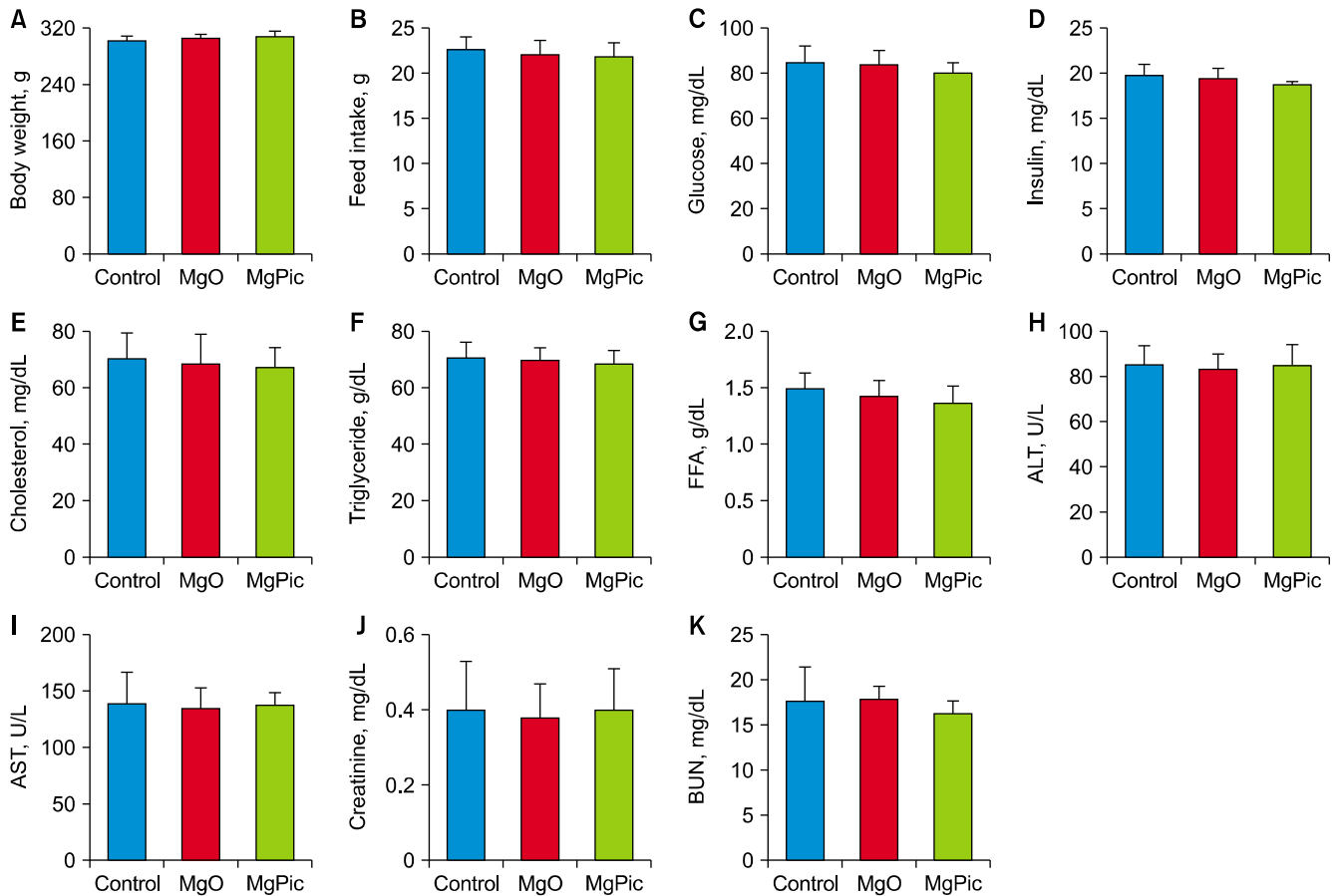
Fig. 1. Effect of magnesium oxide (MgO) and magnesium picolinate (MgPic) on the health and serum biochemistry of the rats. (A) Body weight, (B) feed intake, (C) glucose, (D) insulin, (E) cholesterol, (F) triglyceride, (G) free fatty acid (FFA), (H) alanine aminotransferase (ALT), (I) aspartate aminotransferase (AST), (J) creatinine, (K) blood urea nitrogen (BUN). The data represent mean±standard deviation (n=7 rats per group). ANOVA and Tukey's post hoc tests were used to compare the results among different groups ($P>0.05$ for all).

Table 2. Effects of MgO and MgPic supplementation on body weight, feed intake, and serum biochemical parameters in rats

Parameter	Groups		
	Control	MgO	MgPic
Serum Mg (mg/dL)	2.18±0.07	2.46±0.06*	2.70±0.17*,#
Liver Mg (μg/g)	207.49±5.21	216.34±2.98*	228.45±6.55*,#
Feces Mg (mg/d)	1.71±0.17	1.72±0.13	1.23±0.11*,#
Urine Mg (mg/d)	1.39±0.05	1.38±0.05	1.24±0.08*,#
Mg absorption (mg/d)	8.83±0.51	18.94±1.38*	19.50±1.45*
Mg absorptivity (%)	83.77±0.98	91.65±0.51*	94.05±0.56*,#
Mg retention (mg/d)	7.45±0.54	17.55±1.38*	18.26±1.49*
Mg retentivity (%)	70.58±1.31	84.92±0.92*	88.01±1.15*,#

Values are presented as mean±standard deviation (n=7 rats per group).
The statistical significance of multiple comparisons was tested with ANOVA and Tukey's post hoc test.
*Comparison to the control group ($P<0.05$). #Comparison to the MgO group ($P<0.05$).
Mg, magnesium; MgO, magnesium oxide; MgPic, magnesium picolinate.

DISCUSSION

In this study, we explored how MgPic, an organic Mg form, influenced Mg absorption, retention, and the function of Mg and Ca transporters in the jejunum. We also compared these effects to those of MgO, an inorganic Mg form, to understand the relative efficacy of these sources of Mg. We demonstrated that MgPic positively affected the Mg balance without any detrimental systematic impact. The observed up-regulation of the mineral transporter genes supported our results, revealing that MgPic promoted Mg balance compared to MgO in rats.
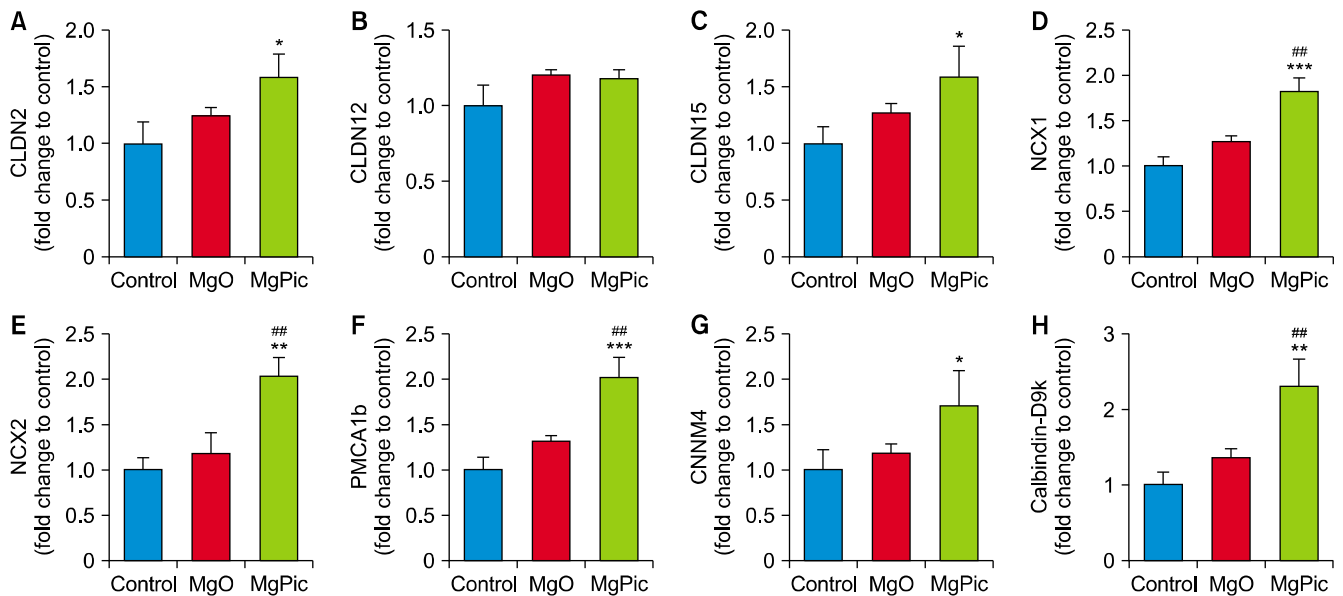
Fig. 2. Effect of magnesium oxide (MgO) and magnesium picolinate (MgPic) on jejunal gene expression in the rats. (A) CLDN2, (B) CLDN12, (C) CLDN15, (D) NCX1, (E) NCX2, (F) PMCA1b, (G) CNNM4, (H) Calbindin-D9k. The data represent mean±standard deviation (n=7 rats per group). ANOVA and Tukey's post hoc tests were used to compare the results among the different groups. *Compared to the control group ($P<0.05$); **Compared to the control group ($P<0.01$); ***Compared to the control group ($P<0.001$); ##Compared to the MgO group ($P<0.01$).

Mg storage in the body is limited, and its retention increases when the body's Mg levels are insufficient. Hence, a high dietary intake of Mg does not affect the Mg balance (Schuchardt and Hahn, 2017). The bioavailability of organic Mg forms is noticeably higher than that of inorganic forms (Blancquaert et al., 2019). A comparison among ten Mg forms reveals that organic Mg salts (acetate, pidolate, citrate, gluconate, lactate, and aspartate) exhibited higher bioavailability than inorganic Mg salts (oxide, chloride, sulfate, and carbonate; Coudray et al., 2005). In addition, Mg gluconate has higher Mg bioavailability than the other nine Mg forms (Coudray et al., 2005). Also, organic Mg compounds (Mg acetyl taurate and Mg malate) markedly elevate Mg bioavailability compared with inorganic Mg compounds (MgO, MgSO₄) (Uysal et al., 2019). Moreover, organic Mg compounds have higher serum and tissue Mg accumulation than inorganic Mg compounds (Uysal et al., 2019). Furthermore, Mg citrate enhances the serum concentration of Mg compared to MgO (Kappeler et al., 2017). Lastly, MgPic likely has a higher solubility and bioavailability than many inorganic and organic Mg forms (Nelson and Komorowski, 2018).

The solubility of PA, which has a tremendous chelating capacity (Seal and Heaton, 1985; Grant et al., 2009), is relatively high in water (Duque et al., 2020). Moreover, PA can enhance the solubility and efficacy of many chelated metals (Grant et al., 2009). For example, zinc picolinate (ZnPic) has higher Zn absorption than gluconate and citrate chelates of zinc (Barrie et al., 1987). In addition, chrome picolinate bioavailability is higher than that of chrome nicotinate or chloride (DiSilvestro and Dy, 2007). Lastly, MgPic synergistically increases the bioavailability of Mg and PA in mammals (Nelson and Komorowski, 2018).

Recently, MgPic has been found to have a greater bioavailability and efficacy than MgO (Orhan et al., 2022). In addition, serum, and liver Mg levels are increased effectively by dietary MgPic supplementation (Orhan et al., 2022). Similarly, in this study, MgPic may have increased serum and liver Mg concentration, compared with MgO, by improving Mg absorptivity and retentivity, owing to the higher water solubility (Nelson and Komorowski, 2018).

Changes in Ca^{2+} and Mg^{2+} luminal concentrations regulate the paracellular diffusion systems (Hardwick et al., 1991). Paracellular cation transport, including that of Mg^{2+} and Ca^{2+} ions, occurs with cation-selective pores formed by tight junction proteins, especially CLDN2, CLDN12, and CLDN15 (Amasheh et al., 2011; Beggs et al., 2017). In this study, as expected, dietary Mg supplemented in the form of MgPic increased the expression of the jejunal CLDN2 and CLDN15 genes. However, the mRNA levels of the CLDN12 gene remained unchanged after Mg supplementation. CLDN12 formation may be required for vitamin D-dependent cation absorption, mainly Ca^{2+}, in enterocytes (Fujita et al., 2008), whereas CLDN2 and CLDN15 formation are required for both cation and water absorption (Rosenthal et al., 2020). The positive association between Mg and water absorption can also explain the disparity in the expression of the gene encoding claudin (Hardwick et al., 1991).

Increased basolateral Mg concentration in Caco-2 cells disrupts passive and transcellular Ca transport mechanisms (Davies et al., 2008). In addition, Mg ions have a high affinity for the calcium-binding domain 2 (CBD2) of NCX1; thus, Mg reduces the Ca-binding capacity of NCX1 (Breukels et al., 2011). However, the organic forms of Mg likely do not inhibit intestinal mineral transporters and, thus, are more easily absorbed, similar to other organic minerals. This improved absorption efficiency enhances Mg bioavailability and ensures that adequate levels are maintained within the body (Coudray et al., 2005; Kappeler et al., 2017; Uysal et al., 2019; Orhan et al., 2022). Subsequently, the Mg concentration in the serum increases, positively regulating the metabolism of vitamin D and calcium; this regulation is crucial for maintaining bone health, muscle function, and overall metabolic balance (Uwitonze and Razzaque, 2018).

Here, we observed that the expression of the genes encoding NCX1, NCX2, PMCA1b, and Calbindin-D9k in the jejunum is more efficiently increased in MgPic-supplemented rats. Similarly, Mg supplements elevate Ca absorption through the active transcellular pathways in the gastrointestinal tract of sheep (Kozakai et al., 2002) and in goats and rats (Kozakai et al., 2004). In addition, during dietary Ca deficiency, the activity of the Ca pump and NCX can be increased in chicken enterocytes as a result of elevated serum 1,25-dihydroxycholecalciferol [$1,25(OH)_2D_3$] levels, which stimulate the expression of the genes needed for Ca absorption (Centeno et al., 2004). Besides, Mg supplements that help accelerate the metabolism of vitamin D positively affect Ca absorption (Uwitonze and Razzaque, 2018). Also, the genes encoding PMCA1b and calbindin, required for intestinal Ca absorption, are up-regulated by increased $1,25(OH)_2D_3$ activity (Areco et al., 2015). Similarly, adding $1,25(OH)_2D_3$ to Caco-2 cells elevates paracellular Ca transporters, including CLDN2 and CLDN12 (Fujita et al., 2008).

In conclusion, we found that organic Mg, such as MgPic, has better bioavailability than inorganic Mg, such as MgO, in rats due to higher absorptivity and retentivity as a consequence of the higher bioavailability of MgPic, and Mg accumulation in both serum and liver was elevated. In addition, MgPic could enhance paracellular and transcellular mineral transport by increasing the expression of the transporter genes. Moreover, Mg supplements, especially organic MgPic, may contribute to Ca absorption by either upregulating the expression of the jejunal Ca transporter genes or increasing the activity of the jejunal Ca transporters. These results indicate that MgPic may help alleviate Mg deficiency. Further investigation is necessary to fully understand the clinical relevance of MgPic and to assess its potential benefits and implications for patient care in a clinical setting. These studies should aim to clarify its efficacy and safety in addressing various health conditions, as well as compare it with other forms of Mg supplements to establish its relative efficacy and value in clinical practice.

AUTHOR DISCLOSURE STATEMENT

The author declares no competing interests.

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