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The bioaccessibility and tolerability of marine-derived sources of magnesium and calcium

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A B S T R A C T

It is generally accepted that mineral deficiencies, including magnesium and calcium, are widespread globally. Dietary supplementation may be an effective approach to combat such deficiencies. However, challenges associated with limited mineral solubility in the digestive system can impede effective dissolution and hinder absorption, leading to deficiency, and undesirable gastrointestinal disturbances including diarrhoea. Seawater is considered to be a rich source of bioactive magnesium, calcium, and 72 other trace minerals. In this study, we examine two different marine-derived multimineral products as potential dietary supplements. Aquamin-Mg, sourced from seawater is rich in magnesium (12%), and Aquamin F, a seaweed-derived multimineral is rich in calcium (32%). Both products also contain a diverse array of over 72 minerals, characteristic of their oceanic origin. Our study comprises two experiments. The first experiment evaluates and compares the solubility of Aquamin-Mg, commercially available magnesium bisglycinate, and Pure Magnesium Bisglycinate (PrizMAG) during *in vitro* digestion using the INFOGEST method. Results demonstrate that Aquamin-Mg exhibits superior solubility than the other magnesium sources during the gastric and intestinal phases, particularly when administered alongside food materials. The second experiment is a randomized, double-blind, placebo-controlled study in a small cohort of healthy older aged adults to assess the tolerability of a combined Aquamin-Mg/Aquamin-F supplement over a 12-week period. The findings indicate that this combination supplement is well-tolerated, with no significant adverse events reported, emphasizing its potential as a means of addressing mineral deficiencies.

1. Introduction

Suboptimal dietary practices, intensive agricultural techniques, and the diminished nutritional value of foods are common factors that contribute to mineral deficiencies [1]. These deficiencies may lead to numerous health issues such as migraines, fatigue, neuromuscular irritability, anxiety, arrythmia and nausea [2–6]. While dietary supplementation is an effective strategy to address insufficiencies and deficiencies [7,8], many magnesium and calcium supplements on the market suffer from limited solubility, tolerability and bioavailability, compromising their efficacy. For instance, magnesium oxide, despite its high elemental magnesium concentration, has low solubility and bioavailability [9], while other sources like magnesium citrate offer better solubility but lower levels of elemental magnesium [10]. These differences arise because magnesium oxide is an inorganic magnesium salt, whereas magnesium citrate is an organic magnesium salt. Studies indicate that organic magnesium salts boast higher solubility and bioavailability compared to inorganic magnesium salts [11,12]. The solubility of a supplement directly affects its dissolution and absorption in the digestive system [13]. Magnesium supplements that do not dissolve effectively, often have limited absorption and tolerability. Unabsorbed magnesium salts exert high osmotic activity in the large intestine, potentially causing undesired gastrointestinal distress [14]. Balancing the need to deliver an adequate amount of elemental magnesium in a supplement that can efficiently dissolve in the gastrointestinal tract, while simultaneously guaranteeing optimal absorption without causing undesired side effects, presents a notable challenge.

Magnesium and calcium are essential minerals that are involved in numerous physiological processes in the human body. Magnesium is required for over 300 enzymatic reactions, including DNA synthesis, energy production, muscle and nerve function [15], while calcium plays a critical role in blood clotting, muscle contraction, nerve function, and bone health [16]. In the USA, the recommended daily allowance (RDA) for magnesium is 320 mg/day and 420 mg/day for adult females and males [17], respectively, while the recommended intake for calcium for both males and females is 1000 – 1200 mg/day [18].

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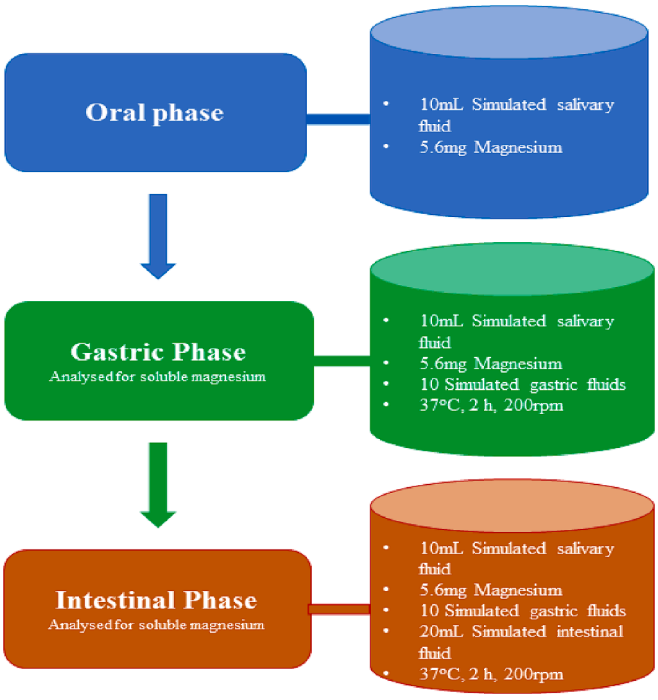

Fig. 1. *In vitro* digestion workflow used to simulate digestion and release of Magnesium from different magnesium supplements.

However, nearly half (48 %) of the US population falls short of the recommended dietary intake of magnesium, while 42 % do not meet the recommended calcium intake from their food [19,20]. In the European Union (EU), the recommended daily intake for magnesium is 300 mg/day for women and 350 mg/day for men [17], while the recommended calcium intake is 1000 mg/day for adult men and women [21]. Similar to the USA, individuals living in the EU also face challenges in meeting the recommended intakes for both calcium and magnesium [22,23].

It has recently been demonstrated that it is possible to extract magnesium from ocean water (Aquamin-Mg). Previous researchers have reported that Aquamin-Mg exhibits high bioavailability, comparable to other magnesium sources [24]. Most notably Aquamin-Mg shows significantly better bioavailability than magnesium oxide and a similar profile to the more bioavailable magnesium chloride. Additionally, the *Lithothamnion sp*. of red seaweed serves as a valuable source of bioavailable calcium known as Aquamin-F. This supplement has previously been shown to have health benefits in terms of bone [25,26], joint [27,28] and digestive health [29–31]. Both products also contain a 'mineral tail' of over 72 minerals, present in small amounts and characteristic of their oceanic origin. The bioavailability of Aquamin-F *in vivo* has been previously established [32]. The combination of Aquamin-Mg and Aquamin-F, known as MMB (Marine Mineral Blend) was chosen as magnesium and calcium deficiencies are common in older adult populations [33,34] and often older adults have reduced ability to digest and absorb nutrients [35]. Aquamin MMB has been tested previously in rodents [36,37].

This study aimed to first evaluate and compare the solubility of Aquamin-Mg with two commonly available commercial magnesium supplements, using the INFOGEST *in vitro* digestion method. The second aim of the study was to evaluate the tolerability of a combination of Aquamin-Mg and Aquamin-F (MMB) in a randomized, double-blind, placebo-controlled study involving a small cohort of healthy older aged adults.

2. Materials and methods

2.1. Solubility of magnesium supplements in water

Magnesium supplement solubility was evaluated at 1 %w/v and 5 % w/v concentration. Fig. 1. shows *in vitro* digestion workflow used to simulate digestion and release of Magnesium from different magnesium supplements. 500 mg (M) and 2500 mg (M) from each of the magnesium supplements were weighed into labelled 50 mL plastic tubes. 50 mL of distilled water was added to the tube and mixed by shaking vigorously. After mixing, the solution was filtered through pre-weighed (M1) 11 μm filter paper (Whatman). The filter paper was dried in the oven at 100 °C overnight. After drying, the filter was placed in a desiccator to cool before weighing (M2). Solubility was calculated as the ratio of soluble fraction [M – (M2-M1)] to initial sample weight (M) and multiplied by 100. Whatman 11 μm filter paper has a molecular weight cutoff as high as 100KDa and intestinal permeability studies are done using molecules ranging between 0.15KDa and 3.35KDa which exhibit high permeability [38]. The molecular weight of the magnesium forms (hydroxide and bisglycinate) in the tested supplements are within this range.

2.2. In vitro digestion of magnesium supplements

The magnesium supplements were subjected to *in vitro* digestion in triplicate according to the INFOGEST protocol as described previously [24,39]. The protocol involved the simulation of oral, gastric, and intestinal digestive phases. All chemicals and enzymes used for the preparation of simulated salivary, gastric, and intestinal fluids were purchased from Sigma Aldrich. The quantity of magnesium digested was calculated based on the NIH Office Of Dietary Supplement. The USA recommended dietary allowance for men (RDA) is set at 420 mg/day, which based on the food bolus used in the digestion protocol was equivalent to 5.6 mg (per body weight of a 75Kg male) of Magnesium from the three sources (commercial magnesium bisglycinate (CMB) (Monarch Nutraceuticals, North Dock Ogden, Utah, United States), Pure Magnesium Bisglycinate (PrizMAG) (ITL Health, Sarnia, Ontario, Canada), and Aquamin-Mg (Marigot Ltd, Carrigaline, Cork, Ireland)). All tested supplements were supplied by Marigot Group Limited, Strand Farm, Curraghbinny, Carrigaline, Co. Cork, Ireland. The oral digestive phase involved the use of simulated salivary fluid containing 15.1 mM KCl, 3.7 mM KH_2PO_4, 13.6 mM $NaHCO_3$, 0.15 mM $MgCl_2(H_2O)_6$, 0.5 mM $(NH_4)_2CO_3$, 1.1 mM HCl and 1.5 mM $CaCl_2(H_2O)_2$ in final volume of 10 mL, adjusted with distilled water. This was followed by the addition of simulated gastric fluid; 6.9 mM KCl, 0.9 mM KH_2PO_4, 25 mM $NaHCO_3$, 47.2 mM NaCl, 0.1 mM $MgCl_2(H_2O)_6$, 0.5 mM $(NH_4)_2CO_3$ to replicate the gastric environment. All chemicals used were laboratory grade. Pepsin (Sigma Aldrich, St. Louis, Missouri, United States) and $CaCl_2$ were included to achieve a final concentration of 2000 U/mL and 0.15 mM in the final volume of the gastric phase (20 mL), respectively. To acidify the mixture to pH 3, hydrochloric acid (HCl, 6 M) was added, followed by water to reach a final volume of 20 mL. Samples were then incubated in a shaking bath (37 °C and 200 rpm) for 2 h. The pH was measured and re-adjusted to pH 3 at 1 h of incubation and 1 mL sample was taken after the 2-hour incubation period and stored in a −20 °C freezer for subsequent elemental analysis.

After the oral and gastric incubation, simulated intestinal fluid (composition: 6.8 mM KCl, 0.8 mM KH_2PO_4, 85 mM $NaHCO_3$, 38.4 mM NaCl, 0.33 mM $MgCl_2(H_2O)_6$) was added together with pancreatin (Sigma Aldrich, St. Louis, Missouri, United States), to achieve a trypsin activity of 100 U/mL, and bile salts (10 mM) (Sigma Aldrich, St. Louis, Missouri, United States). Calcium chloride was added to reach a concentration of 0.6 mM. The pH was adjusted to 7 (NaOH, 1 M) followed by water to reach a final volume of 40 mL. Samples were incubated in a shaking water bath (37 °C and 200 rpm) for an additional 2 h. The pH was measured and adjusted to pH 7 at 1 h of incubation. At the end of the 2-hour incubation period, 1 mL aliquots of each sample were taken and

Table 1
Comparison between the demographics, health status and nutritional status of participants supplemented with placebo or Aquamin MMB.

		Placebo	Aquamin MMB
Demographics	**Gender (no. (%))**		
	Male	7 (24 %)	6 (21 %)
	Female	6 (21 %)	10 (34 %)
	Age (years ± SD)		
	Male	71. 3 ± 7.3	72.8 ± 6.7
	Female	72.0 ± 4.4	75.0 ± 3.8
	Total	71.62 ± 5.92	74.19 ± 5.01
	Race (%)		
	Caucasian	100	100
	Years Education ≥ 13 (no. (%))	10 (77)	15 (94)
Health Status	Weight (kg)	77.9 ± 8.9	71.8 ± 15.2
	BMI (kg/m^2)	27.1 ± 2.6	26.7 ± 4.6
	Alcohol Intake (ml/day)	8.33 ± 9.06	11.33 ± 14.93
	Smoking (no. (%))		
	Non-smoker	6 (46)	10 (62.5)
	Smoker	0 (0)	1 (6.25)
	Former smoker	7 (44)	5 (31.25)
	Physical Activity (MET-min/week ± SD)	3500 ± 2215	6065 ± 5414
Nutritional Status	Caloric Intake (kcal/day)	2393 ± 686.3	2075 ± 604.1
	Dietary Magnesium Intake (mg/day)	400.6 ± 124.0	336.2 ± 101.8
	Dietary Calcium Intake (mg/day)	1081 ± 343.2	1045 ± 580.6

stored in a −20 °C freezer for subsequent elemental analysis. In the digestive simulation involving food material, 5.6 mg of magnesium from each supplement was added to a food sample (In this case, Cottage pie (Dunnes stores, Tralee, County Kerry, Ireland)) to a final weight of 5g [28] and masticated manually prior to being subjected to the digestive simulation. The cottage pie is a mix of Irish beef, onion, gravy, and mashed potatoes and contains 104Kcal/100g, 4.9g/100g total fat, 13.2g/100g total carbohydrate, 1.1g/100g fibre, 3.8g/100g protein, and 0.9g/100g salt. A control was included in the simulation involving pie and magnesium which only contained the food sample and no magnesium supplement. The choice of cottage pie for this experiment was based on the frequency of global consumption of its major ingredients, for example potato is the third most important crop in the world [33] and beef is globally consumed by millions.

2.3. Magnesium quantification using inductively coupled plasma mass spectroscopy

Magnesium concentration of samples taken from the gastric phase and intestinal phase, were analysed using an inductively coupled mass spectrometry system (ICP-MS). Prior to analysis, aliquots taken from the gastric and intestinal phase of digestion were centrifuged (10,000 rpm for 10mins) and digested in a microwave digester (CEM corporation) with a digestant (1:1 water to Nitric acid (65 % Nitric acid), for the digestion 9 mL digestant was added to 1 mL sample in a CEM microwave Teflon tubes). The digestion dissolves the minerals and large molecules present in the sample prior to analysis. The digested samples were filtered and diluted appropriately with diluent containing 2 % and 0.5 % ICP-MS grade nitric acid and hydrochloric acid in ultrapure water (18.2mΩ·cm), respectively. Diluted samples were analysed on the inductively coupled plasma mass spectroscopy instrument (Agilent7800 ICP-MS, Santa Clara, CA 95051, USA). Agilent ICP-MS calibration standards (5, 10, 20, 50, 100, 500, 1000, 2000 ppb, and R square of 0.999) were used for the analysis (Agilent, 5301 Santa Clara, CA 95051, United States).

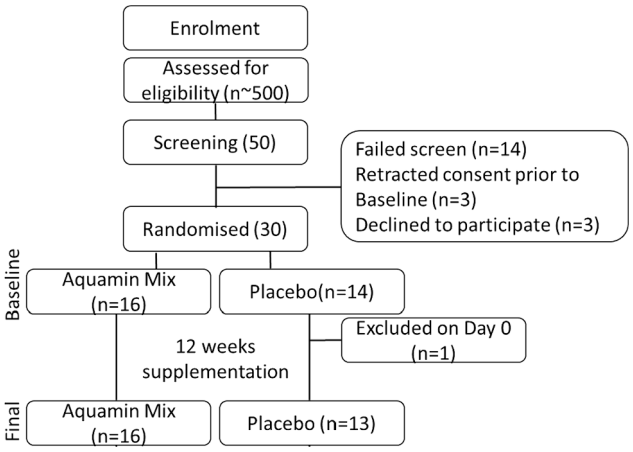

Fig. 2. Flow diagram of subjects/participants.

2.4. In vivo study population

The study protocol (YN001) and all procedures were approved by the Cork Teaching Hospitals Ethics Committee and conducted in accordance with the ICH Guidelines on Good Clinical Practice, and the Declaration of Helsinki. Study participants were male and female between 65 and 82 years of age (Table 1) and were recruited from the carers/loved-ones of patients attending the Assessment and Treatment Centre, St. Finbarr's Hospital Cork, Ireland. Participants were screened for suitability for study inclusion and were scheduled to attend three study visits at the Assessment and Treatment Centre, St. Finbarr's Hospital Cork Fig. 2.

All participants were provided with written information detailing the study involved and all participants provided full written consent prior to the study commencement. A total of 30 participants were randomly assigned to receive either Aquamin MMB or Placebo for twelve weeks. This combination was chosen as magnesium and calcium deficiencies are common in older adult populations [33,34] and often older adults have reduced ability to digest and absorb nutrients [35]. Sixteen subjects received 4 capsules of Aquamin MMB which contained 300 mg Mg^{2+}/day and 235 mg Ca^{2+}/day and fourteen subjects received maltodextrin as placebo. In Europe, the EFSA recommended daily intake for magnesium is 300 mg/day for women and 350 mg/day for men [17]. The magnesium inclusion is based on these recommendations. The EFSA recommended daily intake for calcium is 1000 mg/day for adult men and women [21] and we included calcium at 25 % of this recommendation. Adverse events were self-reported by participants.

2.5. Statistical analyses

Water solubility data are mean ± standard error of three independent water solubility experiments performed for each supplement. *In vitro* digestion data are mean ± standard error of three independent simulations (gastric and intestinal phase) for each supplement. The water solubility and *in vitro* digestion data were analysed in SigmaPlot 12.0 (Systat Software, Inc. SigmaPlot for Windows). Data were analysed using one-way analysis of variance (ANOVA) for treatment groups (different magnesium supplements), following Grubbs test for outliers and verification of normal distribution of data. All data were checked for normal distribution using Shapiro-Wilk test prior to ANOVA. Tukey's post hoc test was used for treatment group comparisons to test for significant differences between variable mean values of percentage of water soluble and *in vitro* digestible magnesium of each magnesium supplements. Differences were significant at $p < 0.05$. The *in vivo* trial data were expressed as mean ± standard deviation (SD). Statistical analysis was carried out using *t*-test. Values of $p < 0.05$ were considered statistically significant.

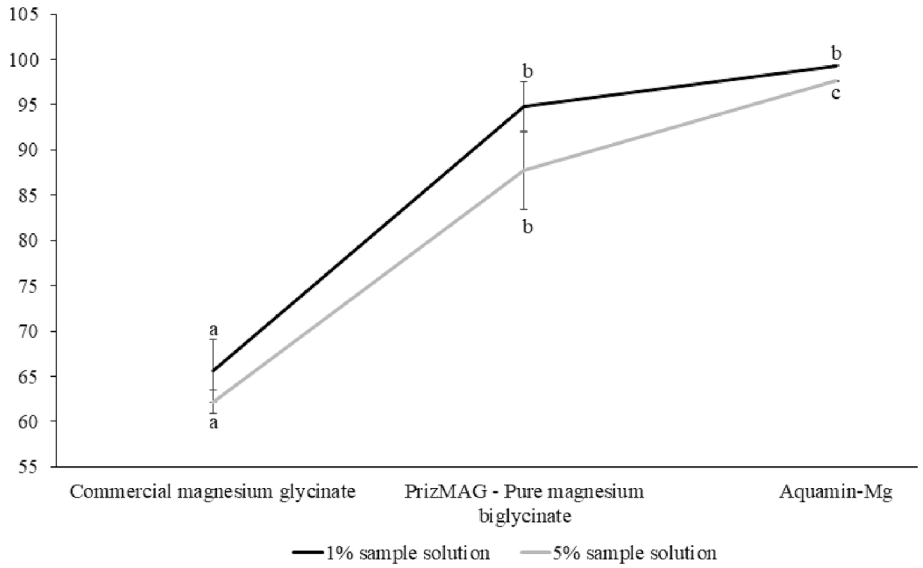

Fig. 3. The solubility of magnesium supplements, commercial magnesium bisglycinate, PrizMAG – pure magnesium bisglycinate and Aquamin-Mg at 1 % and 5 % w/v concentration in water. Samples were dissolved in distilled water, filtered, dried, and weighed, and solubility determined. The y axis demonstrates the percentage solubility of each supplement in water. The x axis indicates the three magnesium supplements being tested. Values are means, with their standard errors represented by vertical bars. N = 3, statistically significant differences determined by ANOVA have a P value < 0.001. Letters a, b, and c indicate statistical difference among sample means.

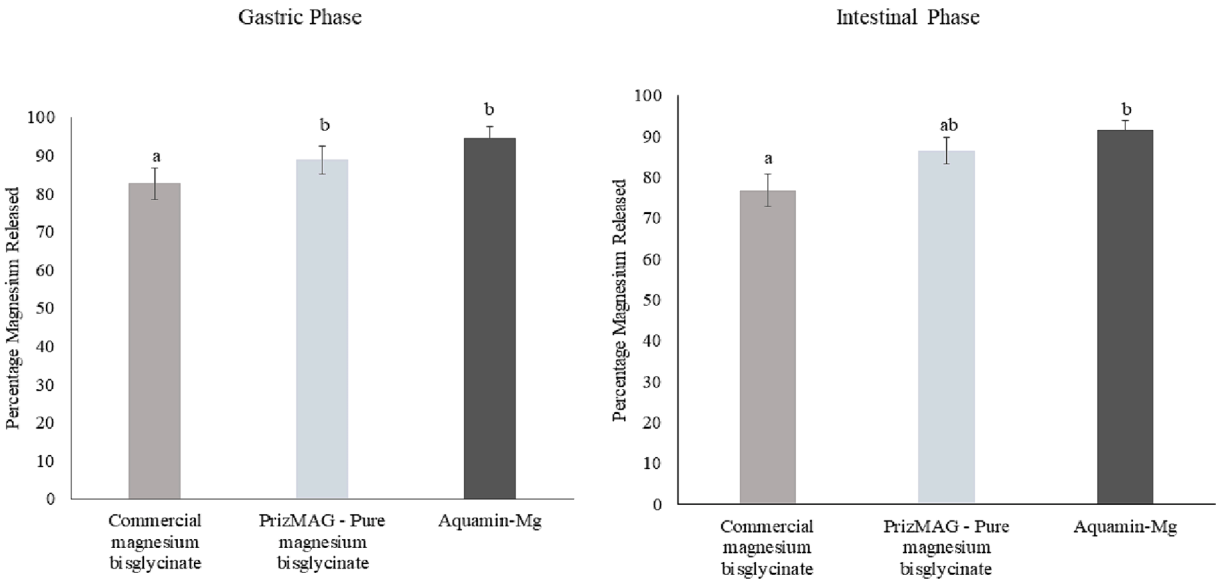

Fig. 4. A and b. Solubility of magnesium supplements, commercial magnesium bisglycinate, PrizMAG – pure magnesium bisglycinate and Aquamin-Mg. The INFOGEST protocol to simulate digestion was used to investigate the solubility of 3 different magnesium samples in the absence of food. Magnesium content was calculated based on RDA. The y axis demonstrates the percentage magnesium released in the gastric and intestinal phase. The x axis indicates the three magnesium supplements being tested. [a-b]Mean values within a row with unlike superscript letters were significantly different (P < 0.05) based on Tukey's post hoc test.

3. Results

3.1. Solubility of magnesium supplements in water

Aquamin-Mg demonstrated the highest solubility at 1 % w/v concentration in water, followed by PrizMag and commercial magnesium bisglycinate, (P < 0.001; Fig. 3). At 5 % w/v concentration in water, Aquamin-Mg demonstrated the highest solubility, followed by PrizMag and commercial magnesium bisglycinate, (P < 0.001; Fig. 3).

3.2. Magnesium recovery from magnesium supplements following in vitro digestion

Aquamin-Mg demonstrated higher solubility in the gastric stage of digestion without food material compared to CMB, but similar to PrizMAG (P = 0.004; Fig. 4**a**). In the intestinal stage of digestion without food material, Aquamin-Mg had higher solubility (94 %±3) compared to CMB (82 %±4) and PrizMAG (89 %±3), (P = 0.018; Fig. 4**b**).

In the gastric phase of digestion with food material, there was no difference in solubility between magnesium supplements, (P = 0.511; Fig. 5**a**). In the intestinal phase of digestion in the presence of food material, Aquamin-Mg had significantly higher solubility compared to

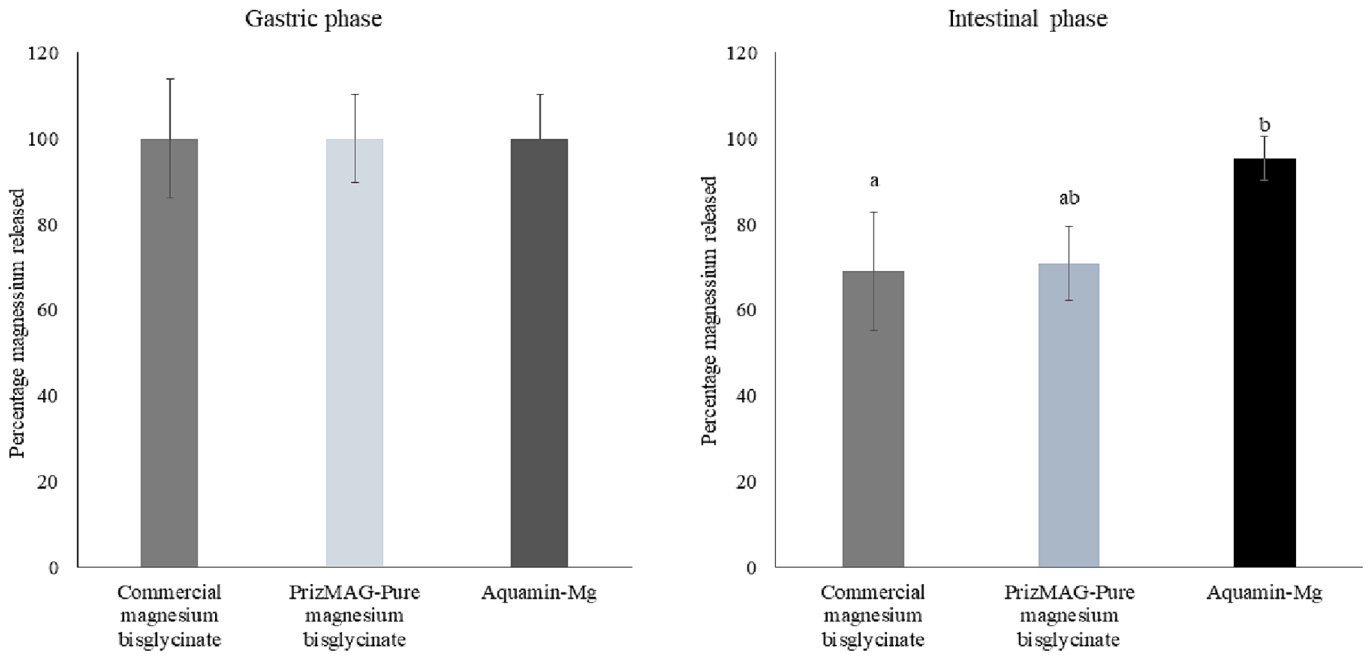

Fig. 5. A and b. Solubility of magnesium supplements, commercial magnesium bisglycinate, PrizMAG – pure magnesium bisglycinate and Aquamin-Mg. The INFOGEST protocol to simulate digestion was used to investigate the solubility of 3 different magnesium samples in the presence of food. Magnesium content was calculated based on RDA. The y axis demonstrates the percentage magnesium released in the gastric and intestinal phase. The x axis indicates the magnesium supplement being tested. [a-b]Mean values within a row with unlike superscript letters were significantly different ($P < 0.05$) based on Tukey's post hoc test.

Table 2
Participant study adherence and adverse events. This table shows the comparison between study adherence and self-reported adverse events of participants supplementing with placebo or Aquamin MMB.

		Placebo	Aquamin MMB
Study Adherence	Days on supplement (days ± SD)	83.9 ± 4.7	84.4 ± 3.8
	Compliance (%)	92.43 ± 5.080	96.79 ± 4.179
Adverse events			
	Gastrointestinal Infection	3	3
	Respiratory	3	1
	Skin	–	2
	Urinary tract infection	2	–
	Other		
	Tired	1	1
	Low mood	–	1
	Vertigo	–	1
	Muscular cramps	3	–
	Melanoma (stage I)	1	–

CMB, ($P = 0.029$; Fig. 5**b**).

3.3. Study adherence and self-reported adverse events

Study adherence and adverse events are shown in Table 2**.** There were no statistical differences in total adverse events, gastrointestinal events, infection events or other events between participants supplemented with Aquamin MMB and those supplemented with placebo.

4. Discussion

A limitation of commercially available magnesium supplements is their low water solubility, limiting potential for effectiveness [12]. This limitation cannot be accounted for by using high-dose magnesium supplements due to potential aggravation of negative gastrointestinal symptoms such as diarrhoea [40]. In this study, Aquamin Mg showed superior solubility in water compared to the two tested commercial magnesium bisglycinates. Also, using the Infogest *in vitro* digestion model, Aquamin Mg was highly soluble compared to a commercial magnesium bisglycinate (CMB) but similar to PrizMAG in both the gastric and intestinal phase of digestion without food. Results of this study showed similar solubility in the gastric phase of digestion among all tested products in the presence of a food material. As the simulation progressed to the intestinal stage of digestion, Aquamin Mg was highly soluble compared to a commercial magnesium bisglycinate but similar to PrizMAG. We then tested the combination of Aquamin-Mg and Aquamin-F (Aquamin MMB) in a population of older healthy adults and demonstrated that this combination supplement was well-tolerated, with no significant adverse events reported.

The solubility of magnesium in the gastrointestinal tract is crucial for its absorption, as minerals must be soluble to be assimilated by the body [12]. Previous assessments of the bioavailability of Aquamin-Mg revealed similar results to $MgCl_2$ [24]. The high solubility of Aquamin-Mg is attributed not only to its natural composition but also to the presence of its multi-mineral tail. Prior research suggests that minerals exhibit enhanced performance when they are combined rather than taken individually [41]. Our findings here demonstrate that magnesium from Aquamin-Mg exhibits higher solubility during the intestinal phase of digestion in the presence of food, compared to CMB but similar to PrizMAG, at equivalent concentrations, suggesting a very bioavailable form of this mineral. Studies on how different forms of magnesium interact with digestion fluids either *in vitro* or *in vivo* remain sparse. There is evidence that magnesium supplements may have different level of bioavailability depending on their method of preparation [42]. It is likely that the higher pH in the intestinal phase of digestion in the presence of food is where interaction with other dietary components is most likely possible and so to have the biggest influence on magnesium solubility [43]. Interactions which can lead to insoluble magnesium containing components are less likely to occur during the gastric phase of digestion. This is due to the higher hydrogen ion content competing with the magnesium for binding sites [44]. Considering these findings and the additional potential health benefits of a multimineral

supplement, Aquamin-Mg emerges as a unique and effective source of magnesium.

Aquamin-Mg having shown promising results in the *in vitro* experiment, was combined with Aquamin F, an Icelandic seaweed derived functional food ingredient, and assessed in older healthy adults. This combination known as Aquamin MMB, was chosen as magnesium and calcium deficiencies are common in older adult populations [33,34]. Our interpretation of the results was that the Aquamin MMB was well tolerated, as no significant differences in adverse events were observed between groups. This is likely due to its high solubility, facilitating mineral absorption by the body, in contrast to unabsorbed magnesium salts known to cause diarrhoea due to intestinal osmotic activity [14]. Additionally, Aquamin MMB may compare favourably with the other supplements tested due to the additional 72-trace minerals it contains. It is known that minerals often synergize, potentially enhancing their bioavailability [41]. Several limitations were noted in this study. In the *in vitro* study, there was a limited range of foods examined, while in the *in vivo* study, limitations included a small sample size, participant age, and lack of bioavailability verification.

In conclusion, our data suggests that Aquamin-Mg has greater solubility than commercial magnesium bisglycinate but similar to PrizMAG – pure magnesium bisglycinate. Further trials will be required to further evaluate the efficacy and bioavailability of Aquamin-Mg *in vivo*.

5. Author statement

Y.N. and C.M.L.S. designed the human study, received funding, interpreted the results and assisted in manuscript preparation. S.O.C. and O.D. designed the *in vitro* study. A.D., S.O.C. and O.D. assisted in data analysis, interpretation of results, and manuscript writing. D.M.O. G. assisted in experimental design, interpretation of results and manuscript preparation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Communication

Bioaccessibility and Bioavailability of a Marine-Derived Multimineral, Aquamin-Magnesium

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Abstract: Introduction: Magnesium is an essential mineral involved in a range of key biochemical pathways. Several magnesium supplements are present on the market and their degree of bioavailability differs depending on the form of magnesium salt used. Aquamin-Mg is a natural source of magnesium, containing 72 additional trace minerals derived from the clean waters off the Irish coast. However, the in vitro bioaccessibility and bioavailability of Aquamin-Mg in comparison with other supplement sources of magnesium has yet to be tested. **Method:** Aquamin-Mg, magnesium chloride ($MgCl_2$) and magnesium oxide (MgO) were subjected to gastrointestinal digestion according to the harmonized INFOGEST in vitro digestion method and in vitro bioavailability tested using the Caco-2 cell model. Magnesium concentration was measured by atomic absorption spectrophotometry (AAS). **Results:** Magnesium recovery from both Aquamin-Mg and $MgCl_2$ was greater than for MgO. Magnesium from all three sources was transported across the epithelial monolayer with Aquamin-Mg displaying a comparable profile to the more bioavailable $MgCl_2$. **Conclusions:** Our data support that magnesium derived from a marine-derived multimineral product is bioavailable to a significantly greater degree than MgO and displays a similar profile to the more bioavailable $MgCl_2$ and may offer additional health benefits given its multimineral profile.

Keywords: Aquamin; multimineral supplement; magnesium bioavailability

1. Introduction

Magnesium is an essential mineral for the human body and is involved in a wide range of crucial physiological processes [1]. Magnesium can be obtained from the diet, being naturally present in foods such as green leafy vegetables, seeds, beans, whole grains, fish and nuts, amongst others. However, dietary magnesium intake has been shown to be insufficient in the Western population due to industrial food processing that reduces the nutrient contents including magnesium, as well as changes in dietary habits [2]. Deficiency in magnesium dietary intake may lead to hypomagnesemia which has been associated with several disorders including diabetes, osteoporosis and cardiovascular disease [3–5]. Early symptoms of hypomagnesemia are non-specific and include loss of appetite, nausea, vomiting, lethargy, fatigue and weakness with more pronounced hypomagnesemia characterised by increased neuromuscular excitability including muscle cramps, tremor, tetany and generalized seizures [6].

The market currently offers various supplement preparations to reach the recommended magnesium daily intake. These supplements differ in the type of magnesium salt used which can be either organic (i.e., magnesium citrate and magnesium aspartate) or inorganic (i.e., MgO and $MgCl_2$), their dosage and bioavailability. For example, magnesium from $MgCl_2$ has high bioavailability equivalent to organic

magnesium supplements such as magnesium lactate and aspartate [7]. Moreover, these three sources of magnesium have significantly greater bioavailability than MgO [7]. Magnesium derived from magnesium hydroxide ($Mg(OH)_2$) (Mablet) has been shown to be absorbed into the circulation and, hence, bioavailable in healthy male adults [8]. In a previous study, the bioavailability of magnesium from formulations containing different combinations of magnesium salts displayed similar bioavailability, however the daily dose of magnesium differed [9].

Aquamin-Mg is a natural source of magnesium in the form of $Mg(OH)_2$ derived from the clean waters off the Irish coast. In addition to magnesium, Aquamin-Mg also contains 72 additional trace minerals (Marigot Ltd., Cork, Ireland, Table 1) with the same profile of Lithothamnion Aquamin which has previously been shown to be a highly bioavailable source of calcium [10].

Here we describe for the first time the in vitro bioaccessibility and bioavailability of Aquamin-Mg in comparison with two commercially available sources of magnesium, $MgCl_2$ and MgO.

Table 1. Mineral composition of Aquamin-Mg. (ppm, parts per million).

Mineral	ppm	Mineral	ppm
Aluminum	461	Molybdenum	1.78
Antimony	<0.5	Neodymium	2.810
Arsenic	1.811	Nickel	<0.5
Barium	1.83	Niobium	<0.5
Beryllium	<0.5	Osmium	0.002
Bismuth	<0.5	Palladium	0.301
Boron	186	Phosphorus	117
Cadmium	0.394	Platinum	0.002
Calcium	23,000	Potassium	88.21
Carbon	10,100	Praseodymium	0.697
Cerium	3.411	Rhenium	0.001
Cesium	0.008	Rhodium	0.010
Chloride	613.4	Rubidium	0.063
Chromium	5.83	Ruthenium	0.135
Cobalt	<0.5	Samarium	0.576
Copper	5.09	Scandium	1.050
Dysprosium	0.829	Selenium	<0.5
Erbium	0.613	Silicon	657
Europium	0.197	Silver	<0.5
Fluoride	1.1	Sodium	1467
Gadolinium	0.770	Strontium	84.7
Gallium	0.163	Sulfur	3335
Germanium	0.020	Tantalum	0.016
Gold	<0.5	Tellurium	<0.5
Hafnium	0.046	Terbium	0.140
Holmium	0.194	Thallium	<0.5
Indium	<0.001	Thorium	0.860
Iodine	9.1	Thulium	0.081
Iridium	0.002	Tin	0.179
Iron	1213	Titanium	18.5
Lanthanum	1.01	Tungsten	2.08
Lead	0.604	Vanadium	16.0
Lithium	<0.5	Ytterbium	0.498
Lutetium	0.116	Yttrium	7.38
Magnesium	404,400	Zinc	2.37
Manganese	486	Zirconium	<0.5
Mercury	0.009		

2. Methods

2.1. Harmonized INFOGEST in Vitro Digestion Protocol

To determine biaccessibility, the amount of magnesium subjected to digestion for each compound was calculated according to the recommended dietary allowance for men (RDA, 420 mg/day). 5.6 mg of magnesium from Aquamin-Mg, $MgCl_2$ and MgO were digested according to the harmonized INFOGEST in vitro digestion method published by Minekus and colleagues [11]. Four to five independent digestions were carried out for each compound (Aquamin-Mg, $MgCl_2$ and MgO). Aquamin-Mg, $MgCl_2$ and MgO were exposed to simulated gastric fluid (composition: 6.9 mM KCl, 0.9 mM KH_2PO_4, 25 mM $NaHCO_3$, 47.2 mM NaCl, 0.1 mM $MgCl_2(H_2O)_6$, 0.5 mM $(NH_4)_2CO_3$). Pepsin and calcium chloride were added to the mixture to achieve a final concentration of 2000 U/mL and 0.075 mM respectively. Hydrochloric acid (HCl, 6 M) was then used to acidify the mixture to pH 3 and water was added to reach a final volume of 20 mL. Samples were then incubated in a stirring water bath at 37 °C and 95 rpm for 2 h. The pH was checked after 1 hour and adjusted if necessary. The simulated intestinal fluid (composition: 6.8 mM KCl, 0.8 mM KH_2PO_4, 85 mM $NaHCO_3$, 38.4 mM NaCl, 0.33 mM $MgCl_2(H_2O)_6$) was then added together with pancreatin (the concentration was based on trypsin activity, 100 U/mL) and bile salts for a final concentration of 10 mM. Calcium chloride was also added to achieve a final concentration of 0.3 mM. Sodium hydroxide (NaOH, 1 M) was used to bring the pH to 7 and the necessary amount of water added to reach a final volume of 20 mL. Samples were incubated in a stirring water bath at 37 °C and 95 rpm for 2 h. The pH was checked after one hour and adjusted if necessary. A control sample containing all reagents included in the digestion protocol except the experimental powders was also subjected to the procedure. Upon completion of the incubation period, aliquots (1 mL) of each sample were frozen in liquid nitrogen. Prior to the analysis, one sample from each treatment was filtered using 0.2 μm cell culture sterile filters. The amount of magnesium recovered from these samples was then compared to non-filtered samples.

2.2. Caco-2 Cell Bioavailability Assay

Caco-2 cells are human epithelial colorectal adenocarcinoma cells that, upon differentiation, express numerous morphological and biochemical characteristics of small intestinal enterocytes. This in vitro model is widely used to study mineral bioavailability from different sources and their transport mechanisms [12].

For Caco-2 bioavailability experiments 60 mg of magnesium derived from Aquamin-Mg, $MgCl_2$ and MgO were subjected to the harmonized INFOGEST in vitro digestion protocol described above (data not shown), and unfiltered samples were used. 60 mg of magnesium was chosen in order to ensure that sufficient concentrations of magnesium could be achieved to perform the Caco-2 experiments. Three independent digestions were carried out for each compound (Aquamin-Mg, $MgCl_2$ and MgO) and these were subsequently used in the Caco-2 bioavailability assay. Caco-2 cells (supplied by the European Collection of Authenticated Cell Cultures (ECACC)) were cultured in Dulbecco's modified eagle's medium (DMEM) supplemented with 1% non-essential amino acids and 10% foetal bovine serum (FBS) and were stored in a humidified incubator at 37 °C and 5% CO_2. For all experiments, cells were seeded at a density of 1×10^5 cells/mL on 6-well Transwell plates with inserts of 24 mm diameter and differentiated for 21 days. Media was changed every other day.

2.3. Bioavailability of Magnesium from Aquamin-Mg, $MgCl_2$ and MgO Using Caco-2 Cells

On the day of the experiment, media was removed from all wells and 1 mL of fresh media was added to the luminal side and 2 mL to the basolateral side of each well. Transepithelial electrical resistance (TEER) was measured to confirm the integrity of the epithelial monolayer. Cells were then incubated with either Aquamin-Mg, $MgCl_2$- or MgO-derived magnesium in the luminal side (concentrations of 25, 50, 100 and 150 μg/mL) for 2 h at 37 °C. Samples from each independent digestion were used in a corresponding independent bioavailability study which was conducted in

triplicate. Two controls were included in the assay, a blank sample, only containing media, and a digest sample, containing all reagents included in the digestion protocol except the experimental powders. At the end of the incubation time, TEER values were recorded again to ensure that the treatments did not have any effect on the integrity of the monolayer. Luminal and basolateral samples were collected and stored at 4 °C. Magnesium concentration of luminal and basolateral samples was measured by atomic absorption spectrophotometry (AAS). Three independent experiments were carried out. Each treatment was randomly assigned and was performed in duplicate.

2.4. Atomic Absorption Spectrophotometry (AAS)

The magnesium content of the digested samples, as well as luminal and basolateral samples was determined by AAS. Samples were diluted in Milli-Q water prior to analysis. Lanthanum chloride (0.1%) was also added to eliminate any phosphate interferences. A commercially available magnesium standard (Spectrosol from BDH Chemicals Ltd., Dublin, Ireland) was used. Standard solutions were prepared using Milli-Q water containing lanthanum chloride (0.1%) and ranged from 0 to 1 mg/L.

2.5. Statistical Analyses

Data are expressed as mean ± standard error of the mean (SEM). Statistical analysis was carried out using the Kruskal–Wallis test, followed by Dunn's multiple comparison test for the digestion study. For the bioavailability study, we calculated the residuals of the data to determine whether there were outliers and statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Bonferroni post-hoc test. Values of $p < 0.05$ were considered statistically significant.

3. Results

3.1. Magnesium Recovery from Aquamin-Mg, $MgCl_2$ and MgO Following In Vitro Digestion

Aquamin-Mg-derived magnesium, showed an in vitro bioaccessibility more similar to highly soluble alternative such as $MgCl_2$ than to MgO (Aquamin-Mg, 122.6 ± 4.1 μg/mL, n = 5; $MgCl_2$, 115.4 ± 6.0 μg/mL, n = 4; MgO, 73.39 ± 10.20 μg/mL, n = 4; in unfiltered samples), which is characterised by low solubility (Figure 1, $p < 0.05$ Aquamin-Mg vs. MgO). To determine whether magnesium was lost during filtration for the Caco-2 cell culture experiments, magnesium concentration in filtered samples was also determined and these were lower for all the treatments relative to unfiltered samples (Aquamin-Mg, 99.3 ± 6.2 μg/mL, n = 5; $MgCl_2$, 92.2 ± 5.6 μg/mL, n = 4; MgO, 64.5 ± 9.3 μg/mL in filtered samples).

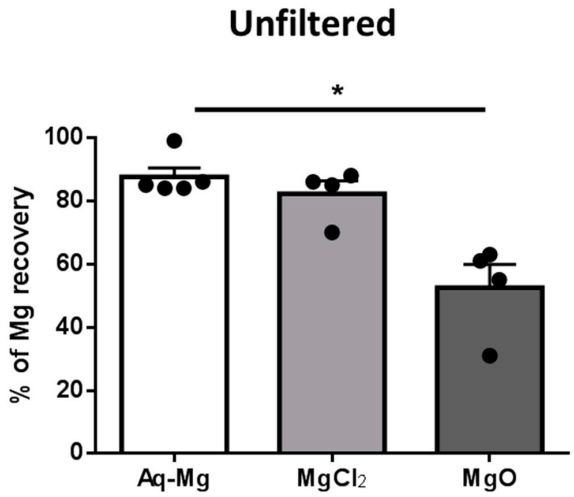

Figure 1. Magnesium recovery from Aquamin-Mg, $MgCl_2$ and MgO following in vitro digestion. The percentage of magnesium recovery from Aquamin-Mg was significantly higher than MgO (* $p < 0.5$, n = 4–5) in unfiltered samples.

3.2. Transepithelial Electrical Resistance (TEER)

As an indicator of cell viability we measured TEER at the start of each experiment and after treatment. The results from the transepithelial resistance measurements confirmed that after 21 days of culture, Caco-2 cells formed an integral monolayer. Moreover, none of the treatments, at any concentration tested, affected epithelial integrity following 2 h incubation at 37 °C (Figure 2).

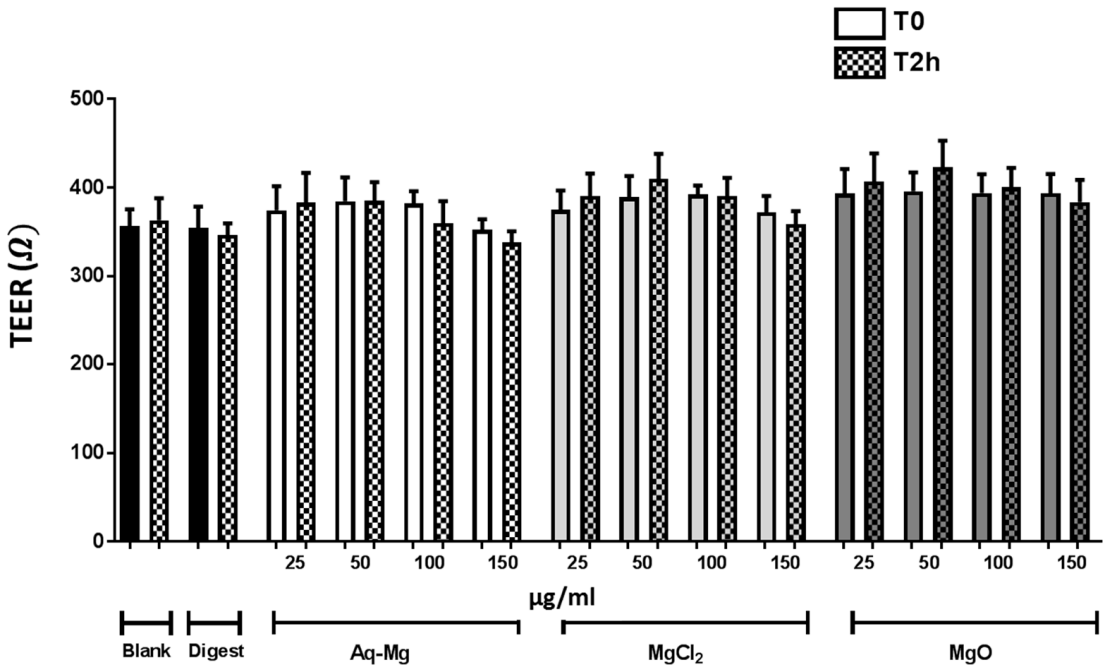

Figure 2. Transepithelial electrical resistance in Caco-2 cells following treatment with Aquamin-Mg, $MgCl_2$ and MgO. At time zero (T0) cells were differentiated in an integral monolayer. Transepithelial electrical resistance (TEER) values confirmed that the treatments did not compromise the integrity of the monolayer at any of the concentrations tested following 2 h incubation (T2h) (n = 3).

3.3. Bioavailability of Magnesium from Aquamin-Mg, $MgCl_2$ and MgO Using the Caco-2 Cell Model

Digestates were added to the luminal compartment. The reduced amount of magnesium from MgO is reflective of the reduced bioaccessibility (Figure 3a). These results are in accordance with the in vitro digestion data (60 mg; data not shown). When the concentration of magnesium was measured in the basolateral chamber, magnesium derived from Aquamin-Mg and $MgCl_2$ showed the same degree of bioavailability at all the concentrations tested, while only a small amount of magnesium from MgO was transported across the epithelium and thus bioavailable. Both Aquamin-Mg and $MgCl_2$ were significantly more bioavailable than MgO at the highest concentration tested (150 µg/mL) (Figure 3b, Table 2).

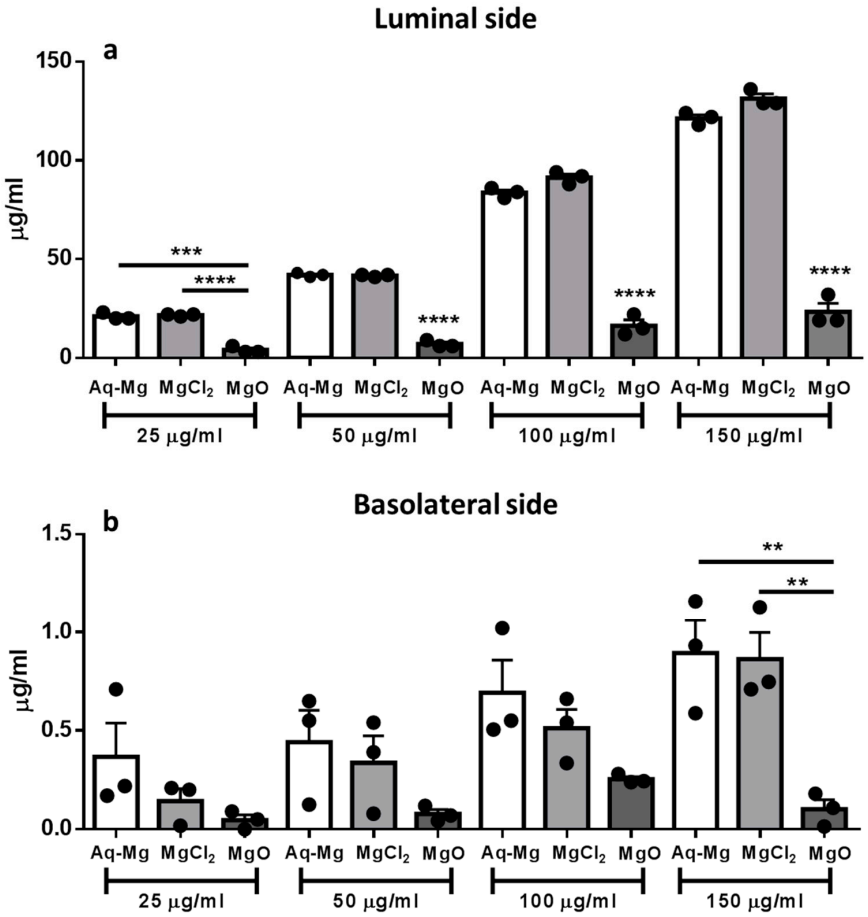

Figure 3. Bioavailability of magnesium from Aquamin-Mg, $MgCl_2$ and MgO using Caco-2 cells. (**a**) Magnesium derived from Aquamin-Mg, $MgCl_2$ and MgO applied into the luminal side. Following in vitro digestion, the amount of bioaccessible magnesium was significantly higher for Aquamin-Mg and $MgCl_2$ compared to MgO (*** $p < 0.001$ for Aquamin-Mg vs. MgO at 25 µg/mL, **** $p < 0.0001$ for $MgCl_2$ vs. MgO at 25 µg/mL and **** $p < 0.0001$ for Aquamin-Mg and $MgCl_2$ vs. MgO at 50, 100 and 150 µg/mL, $n = 3$); (**b**) Magnesium derived from Aquamin-Mg, $MgCl_2$ and MgO measured in the basolateral side after 2 h incubation at 37 °C. At the highest concentration tested, magnesium from Aquamin-Mg and $MgCl_2$ was significantly more bioavailable than MgO (** $p < 0.01$ for Aquamin-Mg and $MgCl_2$ vs. MgO at 150 µg/mL, $n = 3$).

Table 2. Magnesium derived from Aquamin-Mg, $MgCl_2$ and MgO measured in the basolateral side after 2 h incubation at 37 °C expressed as µg/mL ($n = 3$).

Magnesium Source	Sample Concentration (µg/mL)	Basolateral Side µg/mL Mean ± Standard Error of the Mean (SEM)
Aquamin-Mg	25	0.37 ± 0.17
	50	0.44 ± 0.16
	100	0.69 ± 0.16
	150	0.89 ± 0.17
$MgCl_2$	25	0.14 ± 0.06
	50	0.33 ± 0.13
	100	0.51 ± 0.09
	150	0.86 ± 0.13
MgO	25	0.05 ± 0.03
	50	0.08 ± 0.02
	100	0.25 ± 0.01
	150	0.10 ± 0.05

4. Discussion

The aim of these studies was to examine the bioaccessibility and bioavailability of magnesium from Aquamin-Mg compared to $MgCl_2$ and MgO using the Caco-2 cell model. In this model both active saturated and passive non-saturated pathways have been previously identified for magnesium transport [13]. The study from Thongon and Krishnamrain has indeed shown that in Caco-2 monolayers, magnesium transported from the apical to the basolateral side (representing magnesium absorption) against magnesium in the apical solution (representing magnesium concentration) was curvilinear as previously shown in humans [13,14]. Furthermore, the same study has shown that treatment with omeprazole selectively inhibited the non-saturable passive component, without affecting the saturable active component of magnesium transport which was abolished using the Transient Receptor Potential Cation Channel Subfamily M Member 6 (TRPM6) inhibitor Ruthenium Red (RR) [13]. This evidence shows that magnesium can be transported through both a paracellular and a transcellular pathway and that the Caco-2 monolayer is a suitable in vitro model of intestinal magnesium absorption. In the context of our findings, however, we cannot comment on which pathway was responsible for the apical to basolateral transport of magnesium and further research is warranted in order to elucidate these mechanisms. Our results show for the first-time, however, direct evidence that Aquamin-Mg-derived magnesium is highly bioaccessible following in vitro digestion and magnesium is transported across the intestinal epithelium in this well-established in vitro model. Moreover, the degree of bioaccessbility and bioavailability of Aquamin-Mg was comparable to $MgCl_2$ while being superior to MgO.

$MgCl_2$ and MgO represent a high bioavailable and low bioavailable source of magnesium respectively, and our in vitro data are in keeping with in vivo data demonstrating that the mean urinary excretion of magnesium in healthy volunteers was significantly higher for $MgCl_2$ than MgO [7]. Interestingly, in this study, $MgCl_2$ bioavailability was comparable to that of organic magnesium forms such as magnesium aspartate and magnesium lactate [7].

Magnesium in Aquamin-Mg is in the form of $Mg(OH)_2$. However, as well as magnesium, Aquamin-Mg also provides 72 additional trace minerals all derived from sea water (Marigot Ltd., Cork, Ireland, Table 1). In support of $Mg(OH)_2$ as a magnesium supplement, the pharmacokinetic profile of a single oral dose of $Mg(OH)_2$ in healthy male adults showed that the bioavailability of magnesium from $Mg(OH)_2$ was 15% [8]. Moreover, none of the participants recruited reported any side effect following supplementation suggesting that $Mg(OH)_2$ may be a clinically relevant option for oral magnesium supplementation [8]. In a second human study the degree of bioavailability of $Mg(OH)_2$ was compared to other sources of magnesium, including $MgCl_2$ measured as urinary elimination of magnesium [9]. In this study it was found that $Mg(OH)_2$ was required at a higher dose to reach the same level of bioavailability [9].

The solubility of magnesium in the gastrointestinal tract plays a key role in magnesium absorption. Our bioaccessiblity results demonstrate that magnesium from Aquamin-Mg is soluble as $MgCl_2$ and hence potentially available for absorption. Our bioavailability data support that $Mg(OH)_2$, derived from Aquamin-Mg, displays a similar profile and transport characteristics as magnesium derived from $MgCl_2$ at the same concentrations, suggesting that Aquamin-Mg represents a source of magnesium coupled with potential health benefits of a multimineral supplement. Currently, however, as Aquamin-Mg is not formulated as an oral supplement (tablets and capsules), further comparisons with other formulated magnesium supplements were not possible.

Moreover, Aquamin-Mg is composed of multiple minerals and whether these affect its bioaccessibility or bioavailability is difficult to determine.

5. Conclusions

In conclusion, our data suggests that Aquamin-Mg-derived magnesium is bioaccessible and bioavailable to a significantly greater degree than magnesium oxide while displaying a comparable profile to magnesium chloride. Nonetheless, our in vitro results are qualitatively consistent with the clinical study from Firoz and Graber showing that magnesium from $MgCl_2$ has significantly

greater bioavailability than MgO. Further research is warranted to investigate the bioaccessibility and bioavailability of Aquamin-Mg in clinical studies.

Author Contributions: V.D.F., N.P.H., N.M.O. and D.M.O. conceived and designed the experiments. D.M.O. provided Aquamin-Mg. V.D.F. performed the experiments and V.D.F. and N.P.H. analysed the data. V.D.F. and N.P.H. wrote the manuscript; N.M.O., and D.M.O., edited the manuscript.

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From Sea to Supplement

Bioactive Marine Magnesium Multi-mineral Complex

A marine source of magnesium, harvested from the pristine waters of the Irish Sea.

Magnesium
hydroxide and
citrate forms

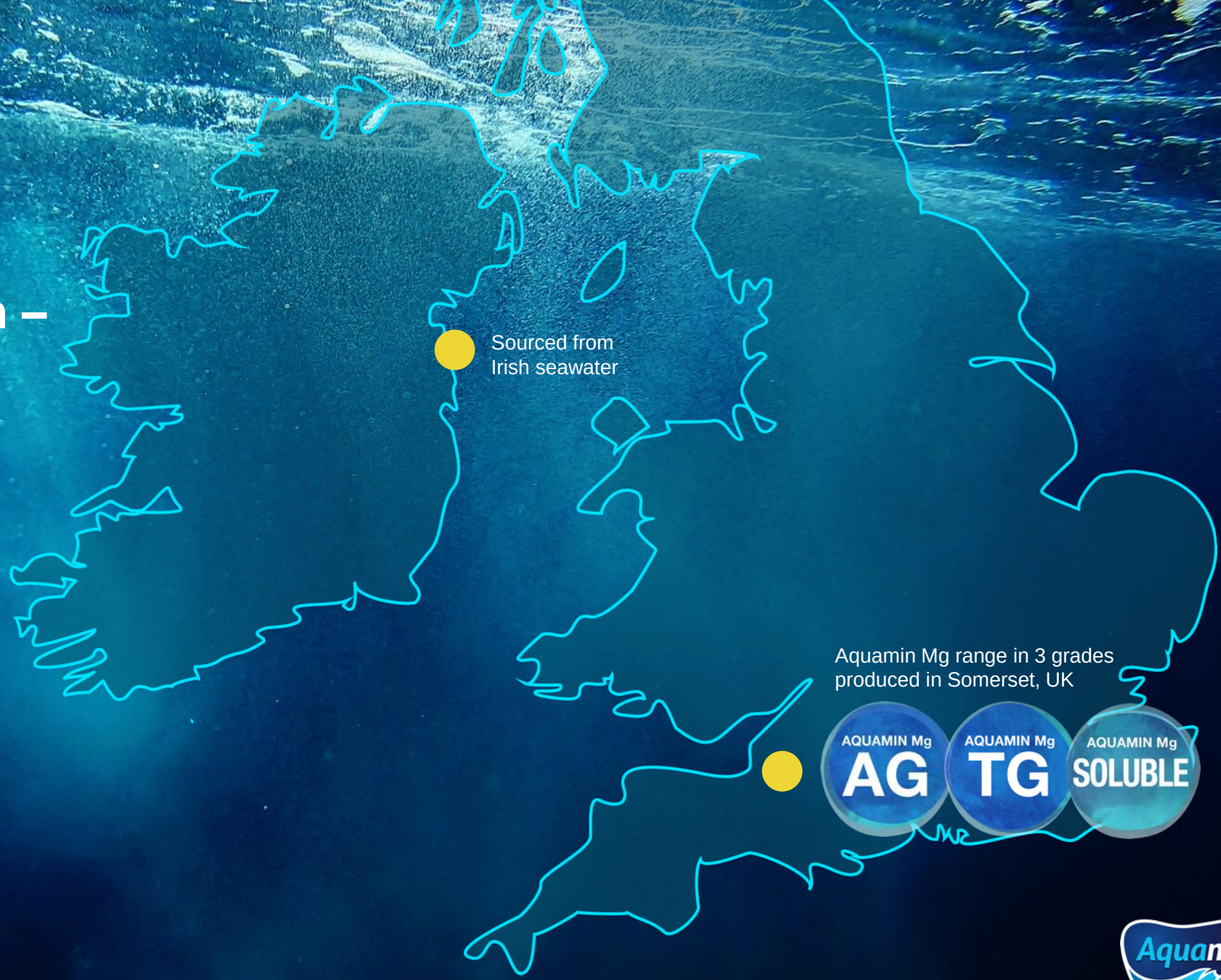
+70 other trace minerals including

Boron	Manganese	Selenium
Iron	Nickel	Aluminium
Zinc	Iodine	Thorium
Potassium	Fluoride	Cerium
Sodium	Cobalt	Carbon
Phosphorous	Silicon	Sodium
Strontium	Chloride	Titanium

Our process

Magnesium in non-dolomite form – Sourced from Irish seawater

- Kosher
- Halal
- Food Grade Facility, ISO & FSSC22000 certified facility, FDA registered



Aquamin Mg grades

Mg %	33
Labelling	Magnesium hydroxide, Arabic gum granulating aid
Appearance	Off-white powder < 500 microns
Applications	Food and Supplements

Mg %	30
Labelling	Magnesium hydroxide, Corn starch granulating aid
Appearance	Off-white powder < 500 microns
Applications	Supplements

Mg %	10-12
Labelling	Magnesium citrate
Appearance	White powder < 300 microns
Applications	Beverage and Supplements

A rich history of scientific and nutritional innovation

1993
Founded by Les Auchincloss (former owner of Biocon) Cork, Ireland.

2002
Established sister company ISM (Icelandic Sea Minerals) Bildudalur, Iceland.

2008
1st Aquamin Science publication – Joint Health.

2020
50 Scientific publications – covering multiple health areas.

2021
Commercial Sales of Aquamin in 40 countries after 25 years!

celebrating 25 YEARS

1996
1st Aquamin commercial food ingredient sales to South Korea.

2007
All Seaweed harvesting Icelandic West Fjords deposit.

2014
Aquamin Mg launched

Magnesium Deficiency – the invisible public health crisis

Magnesium is necessary for the functioning of over 300 enzymes in humans.

Because of chronic diseases, medications, decreases in food crop magnesium contents, and the availability of refined and processed foods, the vast majority of people in modern societies are at risk for magnesium deficiency.

Subclinical magnesium deficiency increases the risk of numerous types of cardiovascular disease, costs nations around the world an incalculable amount of healthcare costs and suffering, and should be considered a public health crisis.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5786912/

Aquamin Mg

Magnesium Recommendations EU

The highest proportions of intakes below the EAR were seen in France (32%) and in the UK (36%).

Summary of Adequate Intakes for Magnesium (EU)

Age	Male	Female
7-11 months	80mg	80mg
1-3 years	170mg	170mg
3-10 years	230mg	230mg
10-18 years	300mg	250mg
+18 years	350mg	300mg

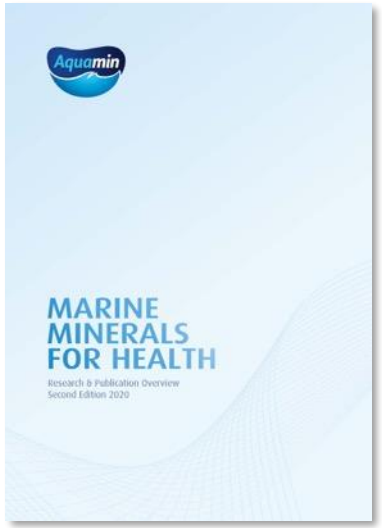
*Including pregnant and lactating women

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3785176/

Why supplementation is important

- Lower nutritional value of intensively farmed fruit and veg
- Loss of magnesium content during food processing
- Many magnesium rich foods are absent from the western diet

Peer reviewed research with our global academic partnerships



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University College Cork, Ireland

Trinity College Dublin
The University of Dublin

UCD DUBLIN

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Ulster University

Aquamin Mg

Supporting physical, energy and wellness systems

Contributes to loosening tight muscles & reduce cramping

Contributes to optimal bone integrity

Helps to avoid hypertension while maintaining muscle & heart health

Stabilizes enzymes used in ATP energy generating reactions

An essential element for optimal nerve transmission correct functioning of CNS

Mg deficiency is associated with poor cognition & cognitive decline

Bioaccessibility and Bioavailability

Bioaccessibility

Amount of magnesium that is potentially available for intestinal absorption following digestion.

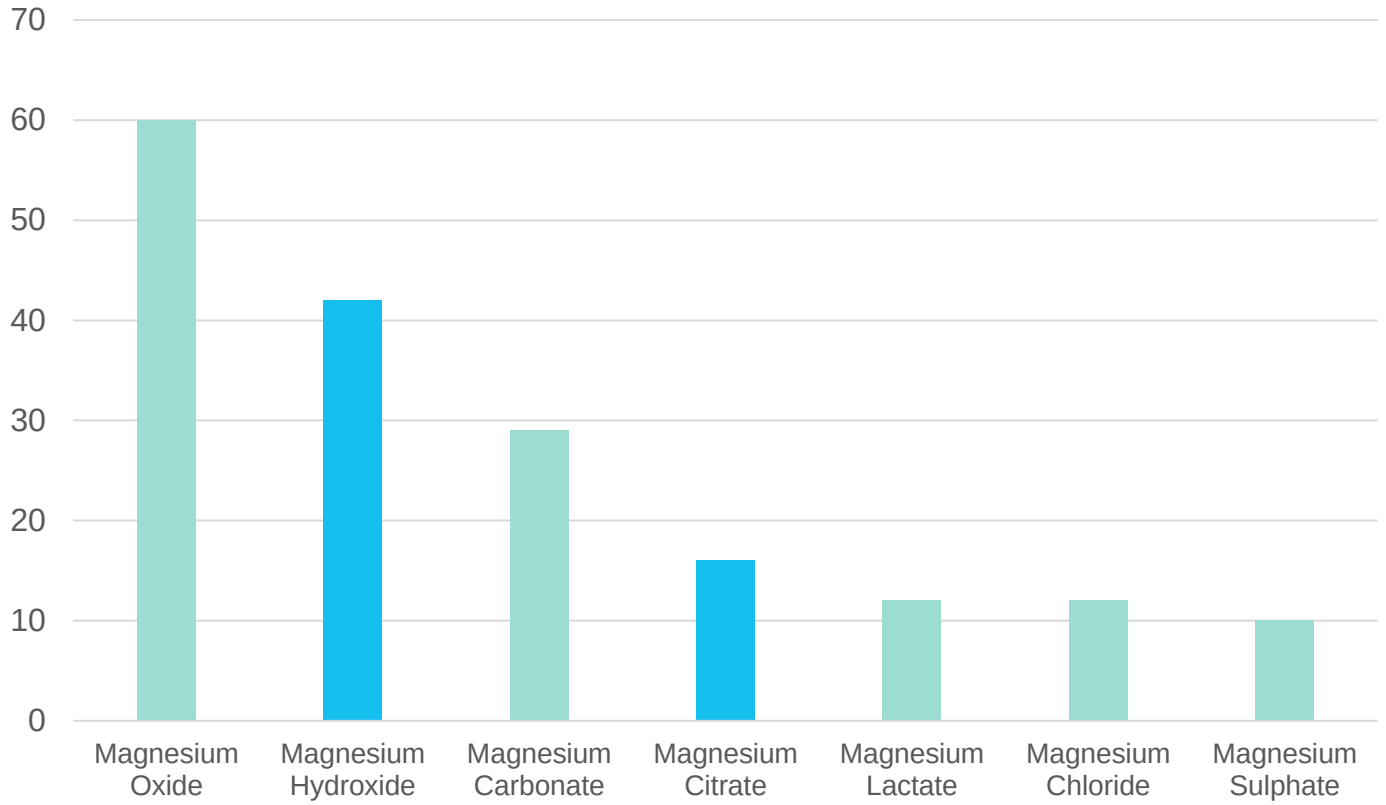
Bioavailability

Amount of magnesium that is absorbed in the intestine and thus available for the body.

Aquamin Mg

Typical Magnesium Supplementation

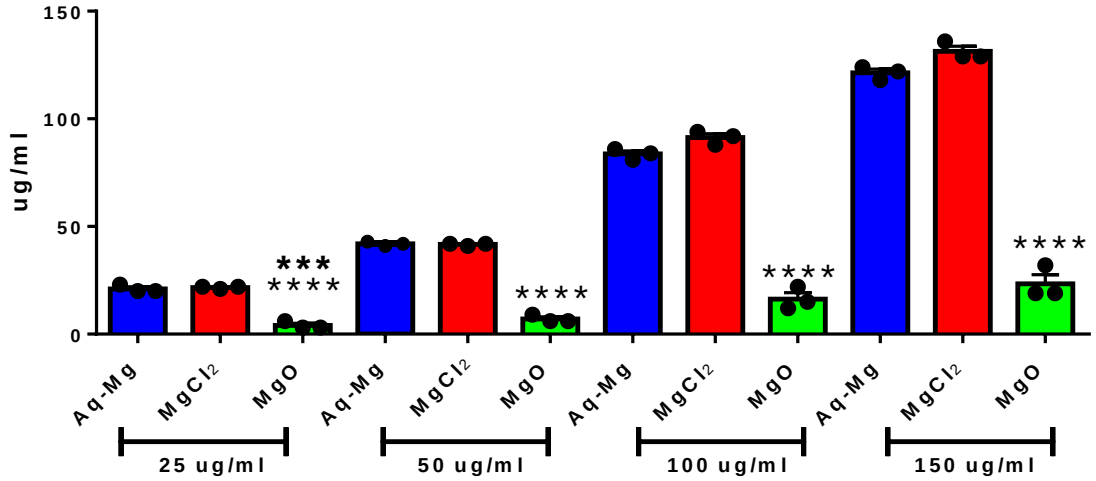
The quality of the magnesium depends not only on its source and magnesium content but also on its bioavailability - the ability of magnesium to be absorbed and utilised by the body.

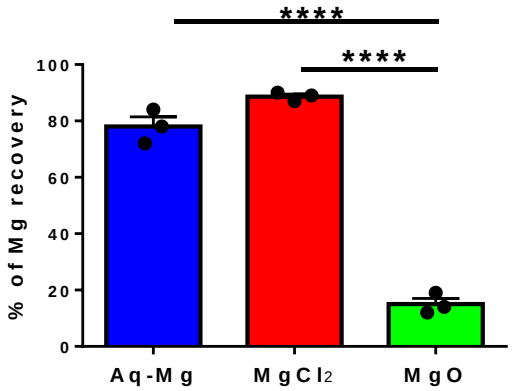


Office Of Dietary Supplements, USA

Aquamin Mg Bioaccessibility

In in-vitro digestion testing Aquamin Mg has been proven as more bio-accessible than Magnesium Oxide.





© Image from Bioaccessibility and Bioavailability of a Marine Derived Multimineral, Aquamin-Magnesium

Valeria D. Felice, Denise M. O'Gorman, Nora M. O'Brien and Niall P. Hyland
Bioaccessibility and Bioavailability of a Marine Derived Multimineral, Aquamin-Magnesium

Aquamin Mg USPs

Harvested from the clear waters of Irish Sea

A marine source of magnesium hydroxide

Also, contains over 70 trace minerals

Bioactive form of Mg & multi-mineral source

Magnesium benefits physical, energy and wellness systems

How we help our customers

In
Innovative Delivery Systems

Flexible

Nutrient Rich, highly bioactive natural minerals for all types of supplement applications.

.

As
Application Support

Solutions

Our technical team can provide hands on support with applications testing and product formulation assistance and development.

Dn
Distribution Network

Partners

Aquamin Mg is a truly global ingredient and is supported by an expanding international distribution network. Contact us today to find your local supply partner.

Thank you

nutrients

MDPI

Communication

Bioaccessibility and Bioavailability of a Marine-Derived Multimineral, Aquamin-Magnesium

Valeria D. Felice [1,2,3,*], Denise M. O'Gorman [4], Nora M. O'Brien [2] and Niall P. Hyland [1,3]

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Abstract: Introduction: Magnesium is an essential mineral involved in a range of key biochemical pathways. Several magnesium supplements are present on the market and their degree of bioavailability differs depending on the form of magnesium salt used. Aquamin-Mg is a natural source of magnesium, containing 72 additional trace minerals derived from the clean waters off the Irish coast. However, the in vitro bioaccessibility and bioavailability of Aquamin-Mg in comparison with other supplement sources of magnesium has yet to be tested. **Method:** Aquamin-Mg, magnesium chloride ($MgCl_2$) and magnesium oxide (MgO) were subjected to gastrointestinal digestion according to the harmonized INFOGEST in vitro digestion method and in vitro bioavailability tested using the Caco-2 cell model. Magnesium concentration was measured by atomic absorption spectrophotometry (AAS). **Results:** Magnesium recovery from both Aquamin-Mg and $MgCl_2$ was greater than for MgO. Magnesium from all three sources was transported across the epithelial monolayer with Aquamin-Mg displaying a comparable profile to the more bioavailable $MgCl_2$. **Conclusions:** Our data support that magnesium derived from a marine-derived multimineral product is bioavailable to a significantly greater degree than MgO and displays a similar profile to the more bioavailable $MgCl_2$ and may offer additional health benefits given its multimineral profile.

Keywords: Aquamin; multimineral supplement; magnesium bioavailability

1. Introduction

Magnesium is an essential mineral for the human body and is involved in a wide range of crucial physiological processes [1]. Magnesium can be obtained from the diet, being naturally present in foods such as green leafy vegetables, seeds, beans, whole grains, fish and nuts, amongst others. However, dietary magnesium intake has been shown to be insufficient in the Western population due to industrial food processing that reduces the nutrient contents including magnesium, as well as changes in dietary habits [2]. Deficiency in magnesium dietary intake may lead to hypomagnesemia which has been associated with several disorders including diabetes, osteoporosis and cardiovascular disease [3–5]. Early symptoms of hypomagnesemia are non-specific and include loss of appetite, nausea, vomiting, lethargy, fatigue and weakness with more pronounced hypomagnesemia characterised by increased neuromuscular excitability including muscle cramps, tremor, tetany and generalized seizures [6].

The market currently offers various supplement preparations to reach the recommended magnesium daily intake. These supplements differ in the type of magnesium salt used which can be either organic (i.e., magnesium citrate and magnesium aspartate) or inorganic (i.e., MgO and $MgCl_2$), their dosage and bioavailability. For example, magnesium from $MgCl_2$ has high bioavailability equivalent to organic

magnesium supplements such as magnesium lactate and aspartate [7]. Moreover, these three sources of magnesium have significantly greater bioavailability than MgO [7]. Magnesium derived from magnesium hydroxide ($Mg(OH)_2$) (Mablet) has been shown to be absorbed into the circulation and, hence, bioavailable in healthy male adults [8]. In a previous study, the bioavailability of magnesium from formulations containing different combinations of magnesium salts displayed similar bioavailability, however the daily dose of magnesium differed [9].

Aquamin-Mg is a natural source of magnesium in the form of $Mg(OH)_2$ derived from the clean waters off the Irish coast. In addition to magnesium, Aquamin-Mg also contains 72 additional trace minerals (Marigot Ltd., Cork, Ireland, Table 1) with the same profile of Lithothamnion Aquamin which has previously been shown to be a highly bioavailable source of calcium [10].

Here we describe for the first time the in vitro bioaccessibility and bioavailability of Aquamin-Mg in comparison with two commercially available sources of magnesium, $MgCl_2$ and MgO.

Table 1. Mineral composition of Aquamin-Mg. (ppm, parts per million).

Mineral	ppm	Mineral	ppm
Aluminum	461	Molybdenum	1.78
Antimony	<0.5	Neodymium	2.810
Arsenic	1.811	Nickel	<0.5
Barium	1.83	Niobium	<0.5
Beryllium	<0.5	Osmium	0.002
Bismuth	<0.5	Palladium	0.301
Boron	186	Phosphorus	117
Cadmium	0.394	Platinum	0.002
Calcium	23,000	Potassium	88.21
Carbon	10,100	Praseodymium	0.697
Cerium	3.411	Rhenium	0.001
Cesium	0.008	Rhodium	0.010
Chloride	613.4	Rubidium	0.063
Chromium	5.83	Ruthenium	0.135
Cobalt	<0.5	Samarium	0.576
Copper	5.09	Scandium	1.050
Dysprosium	0.829	Selenium	<0.5
Erbium	0.613	Silicon	657
Europium	0.197	Silver	<0.5
Fluoride	1.1	Sodium	1467
Gadolinium	0.770	Strontium	84.7
Gallium	0.163	Sulfur	3335
Germanium	0.020	Tantalum	0.016
Gold	<0.5	Tellurium	<0.5
Hafnium	0.046	Terbium	0.140
Holmium	0.194	Thallium	<0.5
Indium	<0.001	Thorium	0.860
Iodine	9.1	Thulium	0.081
Iridium	0.002	Tin	0.179
Iron	1213	Titanium	18.5
Lanthanum	1.01	Tungsten	2.08
Lead	0.604	Vanadium	16.0
Lithium	<0.5	Ytterbium	0.498
Lutetium	0.116	Yttrium	7.38
Magnesium	404,400	Zinc	2.37
Manganese	486	Zirconium	<0.5
Mercury	0.009		

2. Methods

2.1. Harmonized INFOGEST in Vitro Digestion Protocol

To determine biaccessibility, the amount of magnesium subjected to digestion for each compound was calculated according to the recommended dietary allowance for men (RDA, 420 mg/day). 5.6 mg of magnesium from Aquamin-Mg, $MgCl_2$ and MgO were digested according to the harmonized INFOGEST in vitro digestion method published by Minekus and colleagues [11]. Four to five independent digestions were carried out for each compound (Aquamin-Mg, $MgCl_2$ and MgO). Aquamin-Mg, $MgCl_2$ and MgO were exposed to simulated gastric fluid (composition: 6.9 mM KCl, 0.9 mM KH_2PO_4, 25 mM $NaHCO_3$, 47.2 mM NaCl, 0.1 mM $MgCl_2(H_2O)_6$, 0.5 mM $(NH_4)_2CO_3$). Pepsin and calcium chloride were added to the mixture to achieve a final concentration of 2000 U/mL and 0.075 mM respectively. Hydrochloric acid (HCl, 6 M) was then used to acidify the mixture to pH 3 and water was added to reach a final volume of 20 mL. Samples were then incubated in a stirring water bath at 37 °C and 95 rpm for 2 h. The pH was checked after 1 hour and adjusted if necessary. The simulated intestinal fluid (composition: 6.8 mM KCl, 0.8 mM KH_2PO_4, 85 mM $NaHCO_3$, 38.4 mM NaCl, 0.33 mM $MgCl_2(H_2O)_6$) was then added together with pancreatin (the concentration was based on trypsin activity, 100 U/mL) and bile salts for a final concentration of 10 mM. Calcium chloride was also added to achieve a final concentration of 0.3 mM. Sodium hydroxide (NaOH, 1 M) was used to bring the pH to 7 and the necessary amount of water added to reach a final volume of 20 mL. Samples were incubated in a stirring water bath at 37 °C and 95 rpm for 2 h. The pH was checked after one hour and adjusted if necessary. A control sample containing all reagents included in the digestion protocol except the experimental powders was also subjected to the procedure. Upon completion of the incubation period, aliquots (1 mL) of each sample were frozen in liquid nitrogen. Prior to the analysis, one sample from each treatment was filtered using 0.2 μm cell culture sterile filters. The amount of magnesium recovered from these samples was then compared to non-filtered samples.

2.2. Caco-2 Cell Bioavailability Assay

Caco-2 cells are human epithelial colorectal adenocarcinoma cells that, upon differentiation, express numerous morphological and biochemical characteristics of small intestinal enterocytes. This in vitro model is widely used to study mineral bioavailability from different sources and their transport mechanisms [12].

For Caco-2 bioavailability experiments 60 mg of magnesium derived from Aquamin-Mg, $MgCl_2$ and MgO were subjected to the harmonized INFOGEST in vitro digestion protocol described above (data not shown), and unfiltered samples were used. 60 mg of magnesium was chosen in order to ensure that sufficient concentrations of magnesium could be achieved to perform the Caco-2 experiments. Three independent digestions were carried out for each compound (Aquamin-Mg, $MgCl_2$ and MgO) and these were subsequently used in the Caco-2 bioavailability assay. Caco-2 cells (supplied by the European Collection of Authenticated Cell Cultures (ECACC)) were cultured in Dulbecco's modified eagle's medium (DMEM) supplemented with 1% non-essential amino acids and 10% foetal bovine serum (FBS) and were stored in a humidified incubator at 37 °C and 5% CO_2. For all experiments, cells were seeded at a density of 1×10^5 cells/mL on 6-well Transwell plates with inserts of 24 mm diameter and differentiated for 21 days. Media was changed every other day.

2.3. Bioavailability of Magnesium from Aquamin-Mg, $MgCl_2$ and MgO Using Caco-2 Cells

On the day of the experiment, media was removed from all wells and 1 mL of fresh media was added to the luminal side and 2 mL to the basolateral side of each well. Transepithelial electrical resistance (TEER) was measured to confirm the integrity of the epithelial monolayer. Cells were then incubated with either Aquamin-Mg, $MgCl_2$- or MgO-derived magnesium in the luminal side (concentrations of 25, 50, 100 and 150 μg/mL) for 2 h at 37 °C. Samples from each independent digestion were used in a corresponding independent bioavailability study which was conducted in

triplicate. Two controls were included in the assay, a blank sample, only containing media, and a digest sample, containing all reagents included in the digestion protocol except the experimental powders. At the end of the incubation time, TEER values were recorded again to ensure that the treatments did not have any effect on the integrity of the monolayer. Luminal and basolateral samples were collected and stored at 4 °C. Magnesium concentration of luminal and basolateral samples was measured by atomic absorption spectrophotometry (AAS). Three independent experiments were carried out. Each treatment was randomly assigned and was performed in duplicate.

2.4. Atomic Absorption Spectrophotometry (AAS)

The magnesium content of the digested samples, as well as luminal and basolateral samples was determined by AAS. Samples were diluted in Milli-Q water prior to analysis. Lanthanum chloride (0.1%) was also added to eliminate any phosphate interferences. A commercially available magnesium standard (Spectrosol from BDH Chemicals Ltd., Dublin, Ireland) was used. Standard solutions were prepared using Milli-Q water containing lanthanum chloride (0.1%) and ranged from 0 to 1 mg/L.

2.5. Statistical Analyses

Data are expressed as mean ± standard error of the mean (SEM). Statistical analysis was carried out using the Kruskal–Wallis test, followed by Dunn's multiple comparison test for the digestion study. For the bioavailability study, we calculated the residuals of the data to determine whether there were outliers and statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Bonferroni post-hoc test. Values of $p < 0.05$ were considered statistically significant.

3. Results

3.1. Magnesium Recovery from Aquamin-Mg, $MgCl_2$ and MgO Following In Vitro Digestion

Aquamin-Mg-derived magnesium, showed an in vitro bioaccessibility more similar to highly soluble alternative such as $MgCl_2$ than to MgO (Aquamin-Mg, 122.6 ± 4.1 μg/mL, n = 5; $MgCl_2$, 115.4 ± 6.0 μg/mL, n = 4; MgO, 73.39 ± 10.20 μg/mL, n = 4; in unfiltered samples), which is characterised by low solubility (Figure 1, $p < 0.05$ Aquamin-Mg vs. MgO). To determine whether magnesium was lost during filtration for the Caco-2 cell culture experiments, magnesium concentration in filtered samples was also determined and these were lower for all the treatments relative to unfiltered samples (Aquamin-Mg, 99.3 ± 6.2 μg/mL, n = 5; $MgCl_2$, 92.2 ± 5.6 μg/mL, n = 4; MgO, 64.5 ± 9.3 μg/mL in filtered samples).

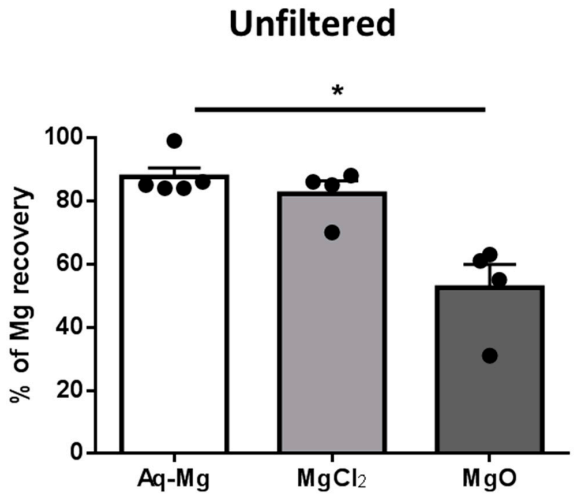

Figure 1. Magnesium recovery from Aquamin-Mg, $MgCl_2$ and MgO following in vitro digestion. The percentage of magnesium recovery from Aquamin-Mg was significantly higher than MgO (* $p < 0.5$, n = 4–5) in unfiltered samples.

3.2. Transepithelial Electrical Resistance (TEER)

As an indicator of cell viability we measured TEER at the start of each experiment and after treatment. The results from the transepithelial resistance measurements confirmed that after 21 days of culture, Caco-2 cells formed an integral monolayer. Moreover, none of the treatments, at any concentration tested, affected epithelial integrity following 2 h incubation at 37 °C (Figure 2).

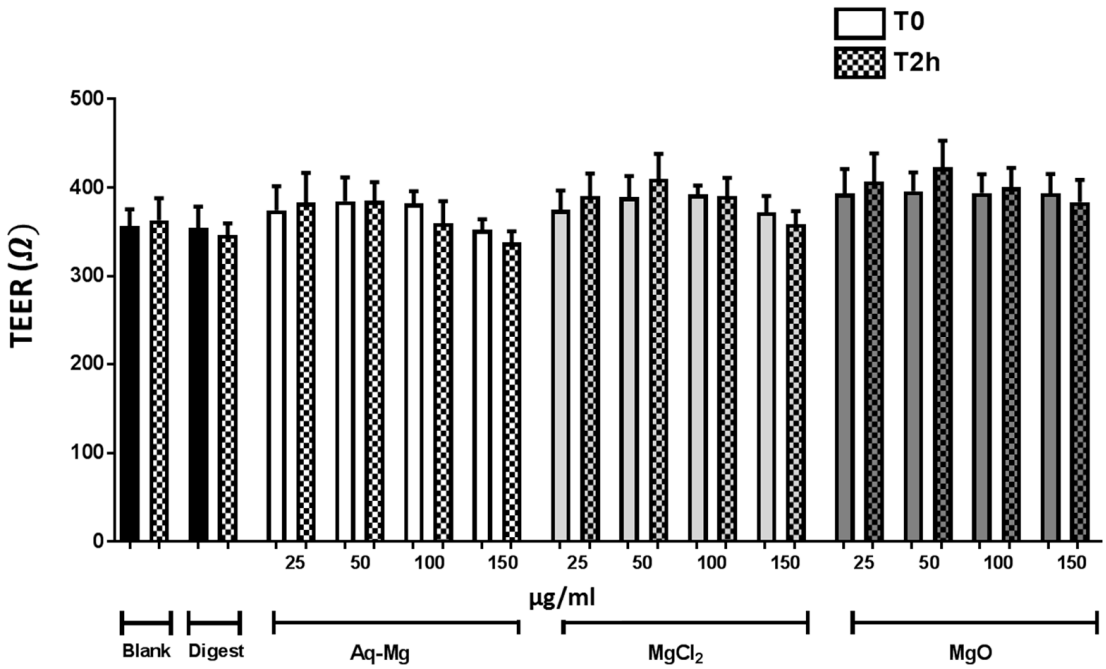

Figure 2. Transepithelial electrical resistance in Caco-2 cells following treatment with Aquamin-Mg, $MgCl_2$ and MgO. At time zero (T0) cells were differentiated in an integral monolayer. Transepithelial electrical resistance (TEER) values confirmed that the treatments did not compromise the integrity of the monolayer at any of the concentrations tested following 2 h incubation (T2h) (n = 3).

3.3. Bioavailability of Magnesium from Aquamin-Mg, $MgCl_2$ and MgO Using the Caco-2 Cell Model

Digestates were added to the luminal compartment. The reduced amount of magnesium from MgO is reflective of the reduced bioaccessibility (Figure 3a). These results are in accordance with the in vitro digestion data (60 mg; data not shown). When the concentration of magnesium was measured in the basolateral chamber, magnesium derived from Aquamin-Mg and $MgCl_2$ showed the same degree of bioavailability at all the concentrations tested, while only a small amount of magnesium from MgO was transported across the epithelium and thus bioavailable. Both Aquamin-Mg and $MgCl_2$ were significantly more bioavailable than MgO at the highest concentration tested (150 µg/mL) (Figure 3b, Table 2).

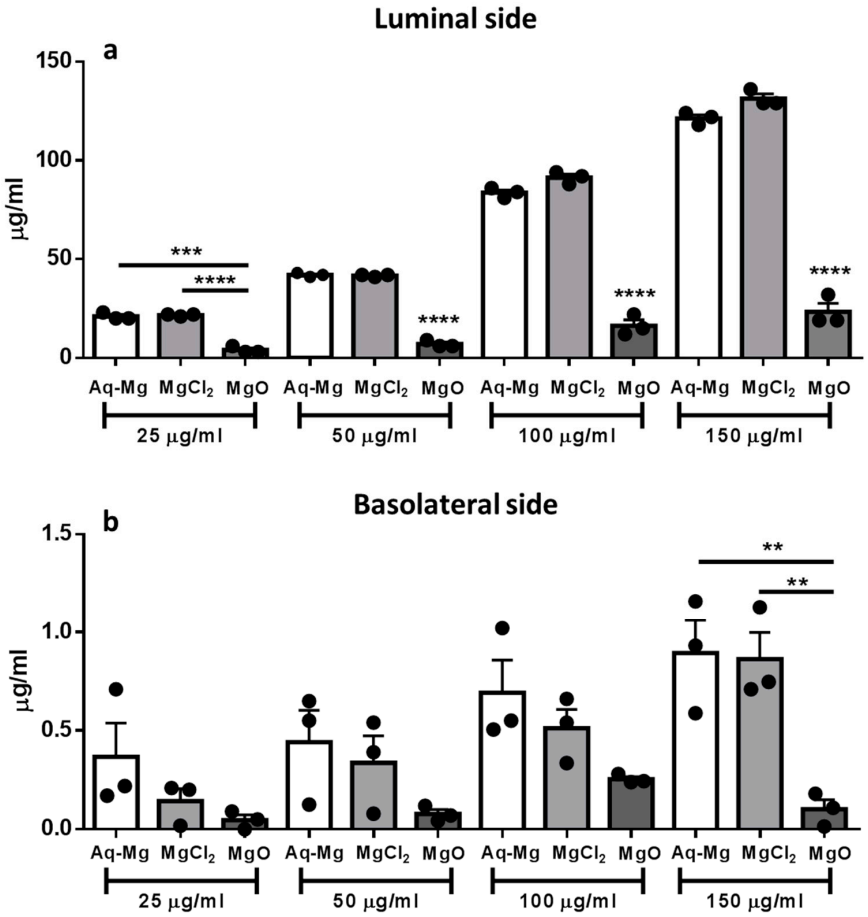

Figure 3. Bioavailability of magnesium from Aquamin-Mg, $MgCl_2$ and MgO using Caco-2 cells. (**a**) Magnesium derived from Aquamin-Mg, $MgCl_2$ and MgO applied into the luminal side. Following in vitro digestion, the amount of bioaccessible magnesium was significantly higher for Aquamin-Mg and $MgCl_2$ compared to MgO (*** $p < 0.001$ for Aquamin-Mg vs. MgO at 25 µg/mL, **** $p < 0.0001$ for $MgCl_2$ vs. MgO at 25 µg/mL and **** $p < 0.0001$ for Aquamin-Mg and $MgCl_2$ vs. MgO at 50, 100 and 150 µg/mL, $n = 3$); (**b**) Magnesium derived from Aquamin-Mg, $MgCl_2$ and MgO measured in the basolateral side after 2 h incubation at 37 °C. At the highest concentration tested, magnesium from Aquamin-Mg and $MgCl_2$ was significantly more bioavailable than MgO (** $p < 0.01$ for Aquamin-Mg and $MgCl_2$ vs. MgO at 150 µg/mL, $n = 3$).

Table 2. Magnesium derived from Aquamin-Mg, $MgCl_2$ and MgO measured in the basolateral side after 2 h incubation at 37 °C expressed as µg/mL ($n = 3$).

Magnesium Source	Sample Concentration (µg/mL)	Basolateral Side µg/mL Mean ± Standard Error of the Mean (SEM)
Aquamin-Mg	25	0.37 ± 0.17
	50	0.44 ± 0.16
	100	0.69 ± 0.16
	150	0.89 ± 0.17
$MgCl_2$	25	0.14 ± 0.06
	50	0.33 ± 0.13
	100	0.51 ± 0.09
	150	0.86 ± 0.13
MgO	25	0.05 ± 0.03
	50	0.08 ± 0.02
	100	0.25 ± 0.01
	150	0.10 ± 0.05

4. Discussion

The aim of these studies was to examine the bioaccessibility and bioavailability of magnesium from Aquamin-Mg compared to $MgCl_2$ and MgO using the Caco-2 cell model. In this model both active saturated and passive non-saturated pathways have been previously identified for magnesium transport [13]. The study from Thongon and Krishnamrain has indeed shown that in Caco-2 monolayers, magnesium transported from the apical to the basolateral side (representing magnesium absorption) against magnesium in the apical solution (representing magnesium concentration) was curvilinear as previously shown in humans [13,14]. Furthermore, the same study has shown that treatment with omeprazole selectively inhibited the non-saturable passive component, without affecting the saturable active component of magnesium transport which was abolished using the Transient Receptor Potential Cation Channel Subfamily M Member 6 (TRPM6) inhibitor Ruthenium Red (RR) [13]. This evidence shows that magnesium can be transported through both a paracellular and a transcellular pathway and that the Caco-2 monolayer is a suitable in vitro model of intestinal magnesium absorption. In the context of our findings, however, we cannot comment on which pathway was responsible for the apical to basolateral transport of magnesium and further research is warranted in order to elucidate these mechanisms. Our results show for the first-time, however, direct evidence that Aquamin-Mg-derived magnesium is highly bioaccessible following in vitro digestion and magnesium is transported across the intestinal epithelium in this well-established in vitro model. Moreover, the degree of bioaccessbility and bioavailability of Aquamin-Mg was comparable to $MgCl_2$ while being superior to MgO.

$MgCl_2$ and MgO represent a high bioavailable and low bioavailable source of magnesium respectively, and our in vitro data are in keeping with in vivo data demonstrating that the mean urinary excretion of magnesium in healthy volunteers was significantly higher for $MgCl_2$ than MgO [7]. Interestingly, in this study, $MgCl_2$ bioavailability was comparable to that of organic magnesium forms such as magnesium aspartate and magnesium lactate [7].

Magnesium in Aquamin-Mg is in the form of $Mg(OH)_2$. However, as well as magnesium, Aquamin-Mg also provides 72 additional trace minerals all derived from sea water (Marigot Ltd., Cork, Ireland, Table 1). In support of $Mg(OH)_2$ as a magnesium supplement, the pharmacokinetic profile of a single oral dose of $Mg(OH)_2$ in healthy male adults showed that the bioavailability of magnesium from $Mg(OH)_2$ was 15% [8]. Moreover, none of the participants recruited reported any side effect following supplementation suggesting that $Mg(OH)_2$ may be a clinically relevant option for oral magnesium supplementation [8]. In a second human study the degree of bioavailability of $Mg(OH)_2$ was compared to other sources of magnesium, including $MgCl_2$ measured as urinary elimination of magnesium [9]. In this study it was found that $Mg(OH)_2$ was required at a higher dose to reach the same level of bioavailability [9].

The solubility of magnesium in the gastrointestinal tract plays a key role in magnesium absorption. Our bioaccessiblity results demonstrate that magnesium from Aquamin-Mg is soluble as $MgCl_2$ and hence potentially available for absorption. Our bioavailability data support that $Mg(OH)_2$, derived from Aquamin-Mg, displays a similar profile and transport characteristics as magnesium derived from $MgCl_2$ at the same concentrations, suggesting that Aquamin-Mg represents a source of magnesium coupled with potential health benefits of a multimineral supplement. Currently, however, as Aquamin-Mg is not formulated as an oral supplement (tablets and capsules), further comparisons with other formulated magnesium supplements were not possible.

Moreover, Aquamin-Mg is composed of multiple minerals and whether these affect its bioaccessibility or bioavailability is difficult to determine.

5. Conclusions

In conclusion, our data suggests that Aquamin-Mg-derived magnesium is bioaccessible and bioavailable to a significantly greater degree than magnesium oxide while displaying a comparable profile to magnesium chloride. Nonetheless, our in vitro results are qualitatively consistent with the clinical study from Firoz and Graber showing that magnesium from $MgCl_2$ has significantly

greater bioavailability than MgO. Further research is warranted to investigate the bioaccessibility and bioavailability of Aquamin-Mg in clinical studies.

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