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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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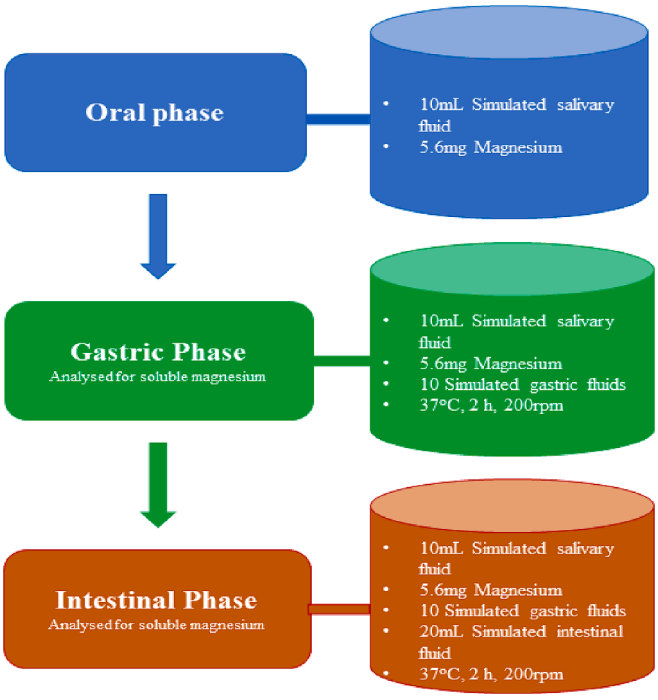

Fig. 1. *In vitro* digestion workflow used to simulate digestion and release of Magnesium from different magnesium supplements.

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).

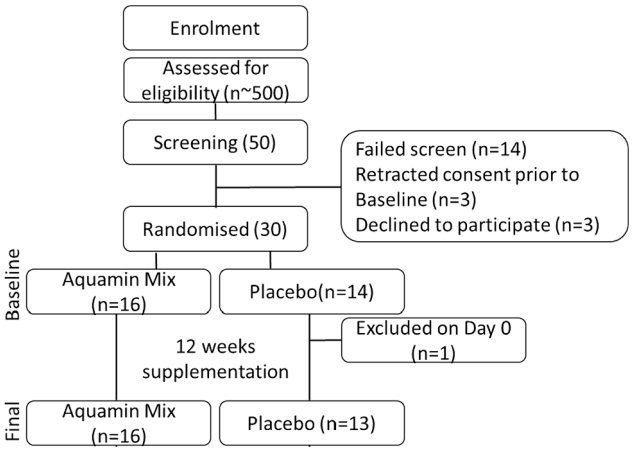

Fig. 2. Flow diagram of subjects/participants.

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.

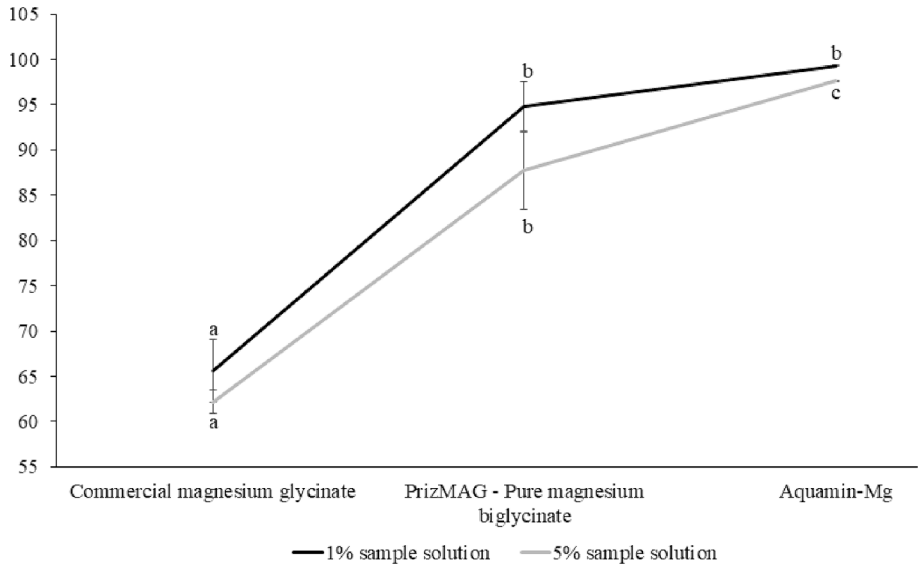

Fig. 3. The solubility of magnesium supplements, commercial magnesium bisglycinate, PrizMAG – pure magnesium bisglycinate and Aquamin-Mg at 1 % and 5 % w/v concentration in water. Samples were dissolved in distilled water, filtered, dried, and weighed, and solubility determined. The y axis demonstrates 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.

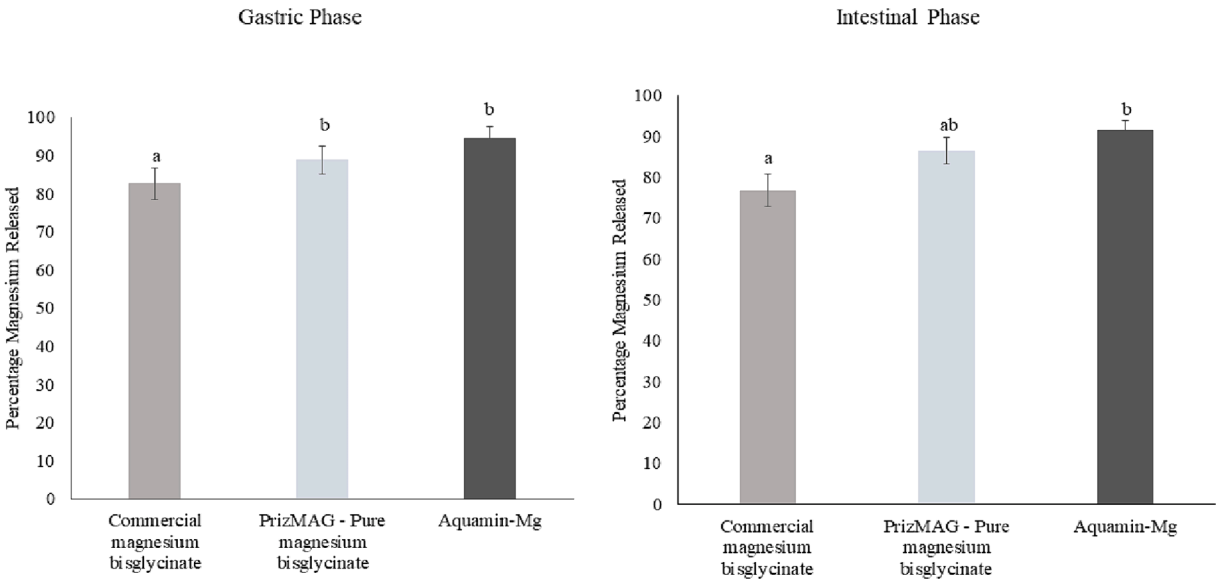

Fig. 4. A and b. Solubility of magnesium supplements, commercial magnesium bisglycinate, PrizMAG – pure magnesium bisglycinate and Aquamin-Mg. The INFOGEST protocol to simulate digestion was used to investigate the solubility of 3 different magnesium samples in the absence of food. Magnesium content 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

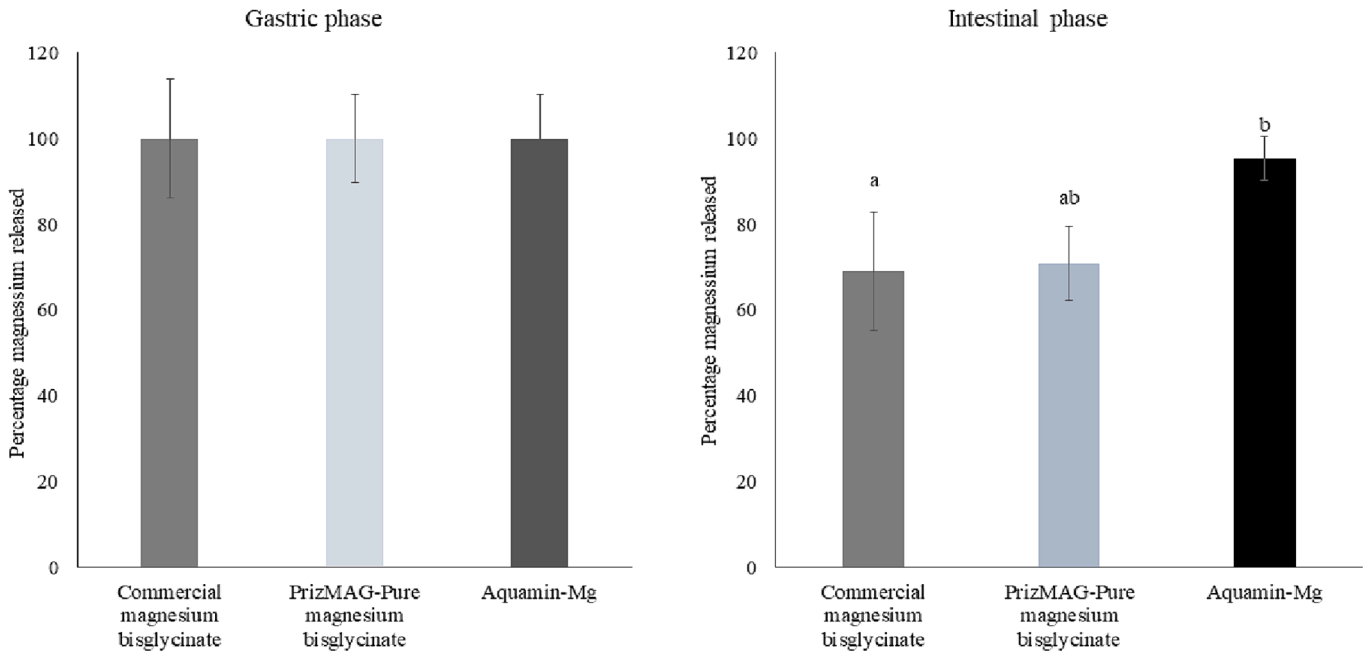

Fig. 5. A and b. Solubility of magnesium supplements, commercial magnesium bisglycinate, PrizMAG – pure magnesium bisglycinate and Aquamin-Mg. The INFOGEST protocol to simulate digestion was used to investigate the solubility of 3 different magnesium samples in the presence of food. Magnesium content 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.

Acknowledgement

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A natural mineral supplement provides relief from knee osteoarthritis symptoms: a randomized controlled pilot trial

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Abstract

Background: This small, pilot study evaluated the impact of treatment with a natural multi-mineral supplement from seaweed (Aquamin) on walking distance, pain and joint mobility in subjects with moderate to severe osteoarthritis of the knee.

Methods: Subjects (n = 70) with moderate to severe osteoarthritis of the knee were randomized to four double-blinded treatments for 12 weeks: (a) Glucosamine sulfate (1500 mg/d); (b) Aquamin (2400 mg/d); (c) Combined treatment composed of Glucosamine sulfate (1500 mg/d) plus Aquamin (2400 mg/d) and (d) Placebo. Primary outcome measures were WOMAC scores and 6 Minute Walking Distances (6 MWD). Laboratory based blood tests were used as safety measures.

Results: Fifty subjects completed the study and analysis of the data showed significant differences between the groups for changes in WOMAC pain scores over time ($p = 0.009$ ANCOVA); however, these data must be reviewed with caution since significant differences were found between the groups at baseline for WOMAC pain and stiffness scores ($p = 0.0039$ and $p = 0.013$, respectively, ANOVA). Only the Aquamin and Glucosamine groups demonstrated significant improvements in symptoms over the course of the study. The combination group (like the placebo group) did not show any significant improvements in OA symptoms in this trial. Within group analysis demonstrated significant improvements over time on treatment for the WOMAC pain, activity, composite and stiffness (Aquamin only) scores as well as the 6 minute walking distances for subjects in the Aquamin and Glucosamine treatment groups. The Aquamin and Glucosamine groups walked 101 feet (+7%) and 56 feet (+3.5%) extra respectively. All treatments were well tolerated and the adverse events profiles were not significantly different between the groups.

Conclusion: This small preliminary study suggested that a multi mineral supplement (Aquamin) may reduce the pain and stiffness of osteoarthritis of the knee over 12 weeks of treatment and warrants further study.

Trial registration: ClinicalTrials.gov number: NCT00452101.

Introduction

Osteoarthritis (OA), also called degenerative joint disease, is a slow destructive process of the joint affecting millions of people worldwide. Although the exact biochemical cause of OA remains unknown, the process usually begins when the joint structures are abnormal or the stress placed on the joint surfaces is unusually high. The secondary inflammation due to progressive articular destruction appears to be localized to the particular joint being affected. Current anti-inflammatory treatments for OA while providing some relief from symptoms are suboptimal and the side effects associated with these treatments; in particular the COX-2 specific NSAID's are becoming increasingly recognized [1,2]. As a result of this, use of alternative treatments and complementary medicines are gaining popularity in the United States among OA sufferers.

Glucosamine, a structural component of cartilage, is recognized as a nutritional supplement by the US FDA but as a pharmaceutical product in most European and Scandanavian countries as well as some Asian and Latin American Countries. Glucosamine has been the subject of many trials [3,4] and is used worldwide as an "alternative" treatment for OA although the extent to which it may provide relief to the symptoms of OA is still unclear. The recent NIH funded Glucosamine/Chondroitin Arthritis Intervention Trial (GAIT) tested the efficacy of glucosamine in providing relief for subjects with symptomatic knee osteoarthritis [5]. In this multi-centre study, glucosamine hydrochloride was tested either alone or in combination with chondroitin sulfate, a gycosaminoglycan that is also a structural component of cartilage and a popular alternative therapy for OA. In this study, the overall group of subjects failed to demonstrate an improvement in symptoms for both the individual and combined treatments possibly as a result of the large (60%) placebo effect observed. Some benefit was observed in a subset of subjects with moderate to severe knee osteoarthritis suggesting that the benefits of these nutraceuticals may be limited to this group.

In addition to glucosamine and chondroitin, other nutraceutical products have been reported to provide relief from OA [6-9]. Cat's claw extract has recently been combined with a mineral based treatment (Sierrasil®) to provide symptomatic relief for a group of mild to moderate OA sufferers. While initially demonstrating some benefit with the cats claw/mineral supplement, Miller and coworkers observed that this benefit was at best temporary for a 1–2 week period [10]. Even though the positive effects were short lived in this subset of OA subjects, growing evidence suggests that minerals may play a role in joint health.

Naturally occurring minerals such as magnesium, copper, manganese, selenium and zinc have shown anti-inflammatory effects in both animal and human studies. In a rat model of osteoarthritis, a deficiency of dietary magnesium was demonstrated to enhance the amount of cartilage damage [11]. Furthermore, increased magnesium in the diet may influence inflammation through reducing the serum level of the pro-inflammatory protein C-reactive protein [12]. The trace element copper is an essential cofactor in enzymes such as the collagen cross-linker lysyl oxidase and the anti-oxidant enzyme super oxide dismutase (SOD) that also requires zinc and manganese as cofactors. Recent evidence has suggested a role for oxidative stress in the pathogenesis of OA whereby an excess of reactive oxygen species arising from an imbalance in the antioxidant status of the joint (such as reduced levels of SOD) may result in cartilage degradation and joint remodeling [13]. Selenium is also an essential co-factor for glutathione peroxidase may have a role in reducing the incidence of osteoarthritic lesion [14,15] Positive roles have also been suggested for trace minerals such as boron and manganese in reducing the symptoms and slowing the pathogenesis of OA [16].

The present study was designed to evaluate the potential for a seaweed-derived multi-mineral supplement to alleviate OA symptoms. The mineral supplement (Aquamin) is derived from the red algae Lithothamnion corallioides which is rich in calcium and magnesium and has a variety of trace minerals (Table 1). The goal of this pilot trial was to gain preliminary data regarding the impact of Aqua-

Table 1: Typical Mineral Composition of Aquamin

Mineral	Dry Salt Weight
Calcium Carbonate	85% (34% calcium)
Magnesium Carbonate	8.5% (2.4% magnesium)
Salt (as chloride)	1.5%
Moisture	3.0%
Trace Minerals**	2.0%
Sulphur	0.7%
Potassium	0.6%
Phosphorus	0.05%
Sodium	0.25%
Manganese	100 ppm.
Zinc	20 ppm
Iron	800 ppm
Iodine	30 ppm
Boron	17 ppm.
Copper	8 ppm.
Cobalt	0.1 ppm.
Selenium	1.0 ppm.

**Aquamin contains a wide spectrum of trace minerals assimilated from sea water of which the minerals outlined in the remainder of the table are a selection.
Aquamin is a natural ingredient and trace mineral levels may vary over time

min, Glucosamine Sulfate, the combination of Aquamin and Glucosamine Sulfate, or Placebo on symptoms and functional abilities of subjects with OA during 12 weeks of treatment.

Materials and methods

Study design

This study was a randomized, double blind, placebo controlled clinical trial with four parallel treatment groups: Aquamin, Glucosamine Sulfate, Aquamin plus Glucosamine Sulfate and Placebo. This trial was performed in compliance with all applicable regulations and guidelines (e.g. International Conference on Harmonization Good Clinical Practices, ICH-GCP, the Declaration of Helsinki, 21CFR50-Protection of Human Subjects, and 21CRF56-Institutional Review Boards) and was approved and continuously reviewed by the Quorum Institutional Review Board (Seattle, WA).

Sample size

Since pretrial information about Aquamin was entirely anecdotal, traditional effect sizes and sample size estimates were not possible. We used our experience from a previous study with similar endpoints to estimate the number of subjects that might be needed for this pilot trial. In our earlier trial, we found that 6 weeks of treatment with glucosamine sulfate and/or hyper-immune milk had a significant impact on WOMAC pain, stiffness and activity scores among 35 OA subjects with 10–13 subjects in each of the 3 treatment arms [6]. Based on this information, we enrolled 15 subjects in each of the four treatment arms (Aquamin, Glucosamine Sulfate, Aquamin plus Glucosamine Sulfate and Placebo) for a total of 70 subjects enrolled in this small pilot trial.

Subjects

This was a single centre study conducted at the Minnesota Applied Research Centre and subjects were recruited by advertising in the Minneapolis, Minnesota area. Subjects of either gender were included if they voluntarily gave informed consent, were ambulatory, 25–75 years old, with normal digestion and absorption, diagnosed with moderate to severe OA of the knee according to their previous medical history and the modified clinical criteria of the American College of Rheumatology [17] and had a Western Ontario and McMaster Universities (WOMAC) Osteoarthritis Index [18] score ≤ 75 in the target knee. The target knee was chosen by physical examination to identify the most severely effected knee for each subject and the cut off point for the WOMAC score was enforced as a means of standardizing the extent of pain and immobility in the small number of subjects recruited for this trial. In order to establish a standardized calcium intake across all treatments, subjects were asked to consume a diet with ~600 mg calcium (e.g. two dairy servings) which was estimated to be 40–60% of their RDI (depending on age) per day.

Exclusion criteria were rheumatoid arthritis, gout, pseudogout, Paget's disease, seizure disorder, insulin dependent diabetes mellitus, uncontrolled hypertension, unstable cardiovascular disease, active hepatic or renal disease, active cancer and/or HIV infection or if they required prescription drugs to control pain; had other clinical trial or experimental treatments in the past 3 months; were pregnant, lactating, or at risk of becoming pregnant; or if they received NSAIDS within 48 hours; intramuscular/systemic corticosteroid injection within 4 weeks; intra-articular corticosteroid injection within 2 months; or inter-articular hyaluronic acid injection within 4 months prior to enrollment.

Each subject received one bottle of 350 two-piece hard shell test article capsules each month. Each bottle (and the capsules inside) appeared identical. Subjects were randomized in blocks of 4 using sequential treatment assignments prepared by the independent consulting statistician. The clinical investigator, statistician, clinic staff and subjects remained blinded throughout the trial to avoid bias. The sequence of the study began with a two week period when subjects were asked to discontinue any prescription or over-the-counter or alternative therapy treatments for osteoarthritis.

At the baseline visit, vital signs were assessed and laboratory tests were performed. Subjects were assessed for WOMAC parameters and a 6 minute walking test was performed. After each month of treatment (at 4, 8 and 12 weeks) the subject's diaries, WOMAC questionnaires, and unused pills were collected, medications/supplements were reviewed, adverse events investigated, vital signs measured, blood was drawn and 6 MWD and WOMAC were measured. Active treatment was completed at week 12 when laboratory tests were repeated. Each subject returned to the clinic at 16, 20 and 24 weeks after their treatment began for monitoring of blood chemistry only. Although there was no reason to expect any beneficial carry over effect on the OA symptoms after removal of the treatments, subjects returned to the clinic every 4 weeks for 12 weeks after termination for blood chemistry readings in order to ensure that there were no adverse consequences on their blood metabolites including their blood calcium levels.

Treatments

The duration of treatment was 12 weeks, administered as three capsules taken with a glass of water, three times per day. The capsules contained Aquamin (267 mg Aquamin F + 167 mg maltodextrin – FCC, USP & NF specifications for 10 Dextrose Equivalent Maltodextrin which was

assumed inert in the capsules) designated A in the results section; Glucosamine sulfate (167 mg D-glucosamine sulfate Potassium salt, Pharmachem Labs NJ, USA + 267 mg maltodextrin) designated GS, Aquamin and Glucosamine (267 mg Aquamin F + 167 mg glucosamine sulfate) designated G+A, or Placebo (434 mg maltodextran) designated PBO in the results section. The rescue medication was acetaminophen, 325 mg, 1–2 tablets every 4–6 hours as needed for intractable pain.

Study measurements and statistical analysis

Joint symptoms were assessed using the Western Ontario and McMaster Universities (WOMAC) Osteoarthritis Index, a validated questionnaire including scores for pain, stiffness and activities as well as a composite (total) score. The WOMAC scores were transformed according to the standard orthopedic formula:

Transformed Score =
100 - (Actual Raw Score × 100/Possible Raw Score) [18]

The values represent "percentage of normal," such that increasing scores reflect improvement and decreasing scores reflect worsening of symptoms. The six minute walking distance (6 MWD) was conducted by marking off a 100-foot distance in an interior hallway and asking subjects to walk as far as they can as quickly as they can over 6 minutes. The total distance was measured and recorded. Adverse effects were assessed by a questionnaire and vital signs/laboratory measurements respectively.

This study was conducted, monitored and audited in compliance with ICH-GCP guidelines and according to the Minnesota Applied Research Center Standard Operating Procedures (SOP's) and the SOP's of certified vendors (e.g. for WOMAC scoring). Subject compliance was assessed at each visit by pill count, interview, and review of the medication diary. Subject data was kept confidential and study records were stored in a locked and secure storage area. An independent statistician used ANOVA for between group comparisons at baseline. ANCOVA (with baseline score as co-variate) were used to assess between-group differences in change over time. Matched pair T-tests were used for within group comparisons of change over time. Data was analyzed under Intent To Treat Last Observation Carried Forward (ITT-LOCF) case condition and statistical significance was set at $p < 0.05$.

Results

Baseline characteristics

Table 2 shows that all four groups were comparable (on average) for number of subjects (N = 15–20), gender (6–11 male; 7–11 female), BMI (30.5 – 32.5), age (58.5 to 60.3 years), WOMAC activity (49.4–63.0) and composite (48.8–63.4) scores and 6 MWD (1323–1427 feet) indicating that the randomization was effective for those parameters. Significant baseline differences were observed between the four groups for WOMAC pain (50.0–67.2) and stiffness scores (40.4–57.8) (p = 0.039 and 0.013 respectively). (Table 3) The finding of baseline differences for the pain and stiffness sub-scores limits the analysis of these data.

Subject attrition after release of the test article

Twenty (20) of the 70 subjects given test article withdrew from the trial prior to completion (29% attrition) and a total of 50 subjects completed the trial (Figure 1). The reasons for subject attrition were spread evenly across the test article administration groups and no pattern was obvious that might suggest withdrawal due to a problem specific to any of the test articles in this trial.

WOMAC

Using an ITT-LOCF analysis, only the improvements in WOMAC pain score differed significantly between the groups over the course of the study (p = 0.009 ANCOVA).

All four groups displayed numerical improvements from baseline to end of treatment for WOMAC scores (Table 3 and Figure 2); however, no significant improvements were demonstrated within groups over time for the placebo or for the combined treatment groups. Within group analysis over time showed that the pain score was significantly improved by 17.5 (P = 0.003 ANOVA) for A and 12.6 (P = 0.003 ANOVA) for GS compared to non significant changes of 2.9 for PBO and 1.9 for G+A (higher score indicates less pain).

Within group analysis over time showed that the activity score was significantly improved by 13.6 (P = 0.010

Table 2: Baseline characteristics (ITT analysis).

Characteristic	N started (completed)	Gender M/F	Mean age (S.D.)	BMI (calc)
PBO	16(9)	6/10	58.9(7.4)	32.4
A	20(15)	11/9	58.5(12.1)	32.5
GS	19(14)	8/11	59.2(8.3)	32.1
G+A	15(12)	8/7	60.3 (9.8)	30.5
p (ANOVA*)	NS	NS	NS	NS

* = ANCOVA comparison between groups

Table 3: Changes in WOMAC Scores at baseline and at the end of the trial Between and Within Groups (ITT-LOCF)

Variable	Pain Baseline	Pain End	Pain baseline – pain end	Stiffness Baseline	Stiffness End	Stiffness baseline – stiffness end	Activity Baseline	Activity End	Activity baseline – activity end	Composite Baseline	Composite End	Composite baseline – composite end
PBO	**50.0**	**52.9**	**-2.9**	**40.4**	**46.3**	**-5.9**	**49.4**	**56.4**	**-7.0**	**48.8**	**54.8**	**-6.1**
SD	22.9	21.4	19.9	17.4	25.3	18.3	23.1	24	18.4	21.8	22.7	17.6
Sig.*			NS			NS			NS			NS
A	**56.8**	**74.3**	**-17.5**	**44.4**	**65**	**-20.6**	**58.8**	**72.4**	**-13.5**	**57.3**	**72.2**	**-14.9**
SD	15.8	17.6	22.7	17	17.5	26.1	16.2	16.8	21.3	15.3	16.6	21.4
Sig.*			.0.003			0.002			0.01			0.006
GS	**60.6**	**72.9**	**-12.6**	**51.3**	**61.8**	**-10.5**	**60.1**	**70.7**	**-10.6**	**59.4**	**70.2**	**-10.9**
SD	14.8	17.6	16.3	13.8	19.8	24.0	13.9	18.4	15.4	13.2	17.6	15.6
Sig.*			0.003			NS			0.008			0.007
G+A	**67.2**	**69.1**	**-1.9**	**57.8**	**64.1**	**-6.3**	**63**	**68.6**	**-5.6**	**63.4**	**68.3**	**-4.9**
SD	13.3	16.9	14.0	15.7	17.6	17.7	8.7	13.1	11.0	8.1	12.9	101
Sig.*			NS			NS			NS			NS
p(ANCOVA)	0.039#		0.009	0.013#		NS	NS#		NS	NS#		NS

*Sig. = significance by within group paired t-test; 2-tailed; ANCOVA comparison between groups with baseline measure as covariate.
= ANCOVA comparison between groups showing any differences at baseline for these parameters.

ANOVA) for A and 10.6 (P = 0.008 ANOVA) for GS compared to non significant changes of 7.0 for PBO and 5.6 for G+A.

Within group analysis over time showed that the composite (total) WOMAC score was significantly improved by 14.9 (P = 0.006 ANOVA) for A and 10.8 (P = 0.007 ANOVA) for GS compared to non significant changes of 6.1 for PBO and 4.9 for G+A.

Of interest, the Aquamin group also displayed a significant improvement over time for stiffness score (20.6, p = 0.002) compared to non significant changes of 10.5 for GS, 5.9 for PBO and 6.3 for G+A.

Subject consumption of rescue medication

No significant differences were found between the groups in the amount of rescue medication consumed over the course of the experiment (Table 4).

Six minute walking distance (6 MWD)

The distance covered during a 6 minute timed walk was significantly improved over time on treatment within the Aquamin group (+101 feet, p = 0.001, Table 5). The glucosamine group also demonstrated a significant improvement in 6 MWD over time on treatment (+56 feet, p = 0.030). No significant improvements were demonstrated within groups over time for the placebo group and surprisingly for the combined treatment group.

Adverse effects

All treatments were well tolerated. Table 6 shows that a total of 51 of the 70 subjects given test product (TA) reported eighty-eight (88) adverse effects (AE) but only 7 of the 88 AE (8%) were considered at least possibly related to the TA treatment. These AE were distributed somewhat evenly across the groups: 1 on PBO, 3 on A, 2 on GS and 1 on G+A and none were considered definitely related to the TA (Table 6). Most of the adverse effects (31/88; 35%) were related to musculoskeletal complaints and these were mainly reports of increased knee pain (n = 19/88; 22%). All AE completely resolved or returned to baseline.

Discussion

This trial was designed as a preliminary pilot trial to investigate the potential of a marine derived multi-mineral supplement to reduce symptoms of moderate to severe knee osteoarthritis. The dose of the mineral supplement Aquamin was determined based on previous anecdotal experience and a rigorous Intent to Treat – Last Observation Carried Forward statistical analysis was used to compare the four treatment groups: Aquamin, Glucosamine Sulfate, Combination of Aquamin and Glucosamine Sulfate, or placebo.

These results were confounded because the WOMAC pain scores were significantly different between the groups at baseline and, therefore, these improvements need to be viewed with caution because further study is warranted. In general, significant differences were found between groups for pain scores after 12 weeks of treatment. Within groups over time, the Aquamin treatment group showed significantly improved WOMAC pain, stiffness, activity and composite scores over the course of the 12-week treatment. The glucosamine sulfate treatment group also showed significant improvements over time on treatment for the pain, activities and composite scores (but not for the stiffness scores); however, no significant improve-

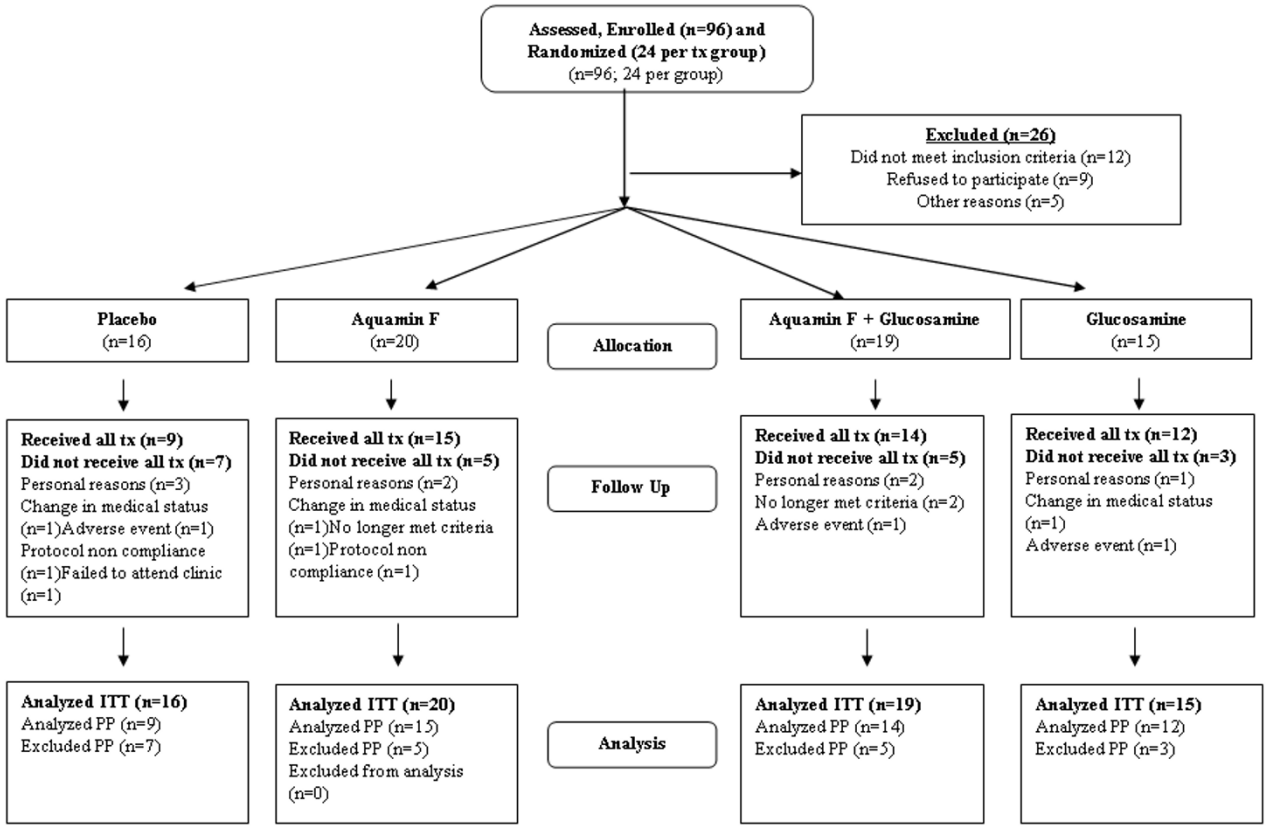

Figure 1
Trial flow chart.

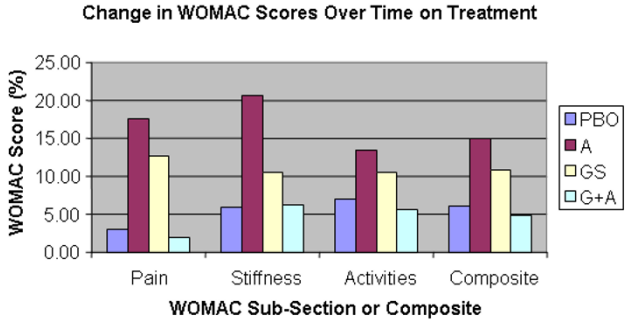

Figure 2
Percent change in WOMAC scores from baseline over 12 weeks of treatment.

Table 4: Consumption of rescue medication.

Group	Month 1	Month 2	Month 3	Total
Placebo	39(38)*	27(27)	30(33)	89(88)
A	45(62)	19(22)	23(31)	87(96)
GS	25(20)	29(38)	31(50)	84(103)
G+A	26(55)	26(34)	27(46)	63(112)

*Mean (SD). Group comparisons of rescue medication were non-significant (Kruskal-Wallis one-way non-parametric analysis of variance) at all time periods and for total medications

Table 5: Changes in 6 MWD Between and Within Groups Over 12 weeks of treatment.

Variable	Baseline 6 MWD	End 6 MWD	Baseline-End 6 MWD
PBO	**1323.4**	**1331.9**	**-8.4**
SD	226.1	250.2	109.3
Sig.*			NS
A	**1427.5**	**1528.8**	**-101.3**
SD	225.6	252.3	121.5
Sig.*			0.001
GS	**1410.1**	**1456.8**	**-55.7**
SD	246.1	256.1	103
Sig.*			0.03
G+A	**1363.1**	**1378.1**	**-15**
SD	253.7	253	126.6
Sig.*			NS
p (ANCOVA)			NS

*Sig. = significance by within group paired t-test; 2-tailed; ANCOVA comparison between groups with baseline measure as covariate

ments were found over time on treatment for subjects in the Placebo group or, surprisingly for subjects in the Combination treatment group.

No baseline differences were observed among the four groups for the 6 minute walking distances. Over time on treatment, the Aquamin and glucosamine groups demonstrated significant improvements in 6 minute walking distances (101 feet, p = 0.001 and 56 feet, p = 0.030, respectively). This was an improvement of 7% and 3.5% respectively over their baseline walking distances. No significant differences were found for the walking distances measured for the placebo group and surprisingly for the combined treatment group. Although, these distances appear to be small, our subjects with severe OA indicated that the ability to walk even a little bit further was important to them.

The main limitations of this study were its short duration (12 weeks), lack of assessment for remnant effects after treatment stopped and limited sample size (15 subjects per treatment arm). Glucosamine sulfate has been shown to provide a benefit over a longer course of treatment [4] and its efficacy may have been under demonstrated within this 12 week study period. Additional study of longer treatments in a greater numbers of subjects would be helpful to verify the treatment effect for Aquamin and to explore the lack of any treatment effect for the combination of Aquamin and Glucosamine Sulfate in this small pilot trial. Although these products are unlikely to have reacted in the tablet form it is interesting to speculate about a possible dietary interaction, possibly related to the very basic nature of Aquamin (pH 10) compared to the acidity of the Glucosamine Sulphate (pH of 3.5 to 5), and the requirement for this to ionize in the stomach to be effective.

Aquamin is composed of multiple minerals and the 'active ingredient' for the complex is difficult to determine. A number of the minerals in Aquamin may have anti-inflammatory and anti-oxidant properties which might directly and/or indirectly influence the efficacy of

Table 6: Adverse effects

Item	Total	GS	A	PBO	G+A
Number of Subjects with AE	51	12	12	14	13
HEENT	25	6	8	6	5
Respiratory/Pneumonia	1	0	0	0	1
Cardiovasular/Hypertension	1	0	0	0	1
Gastrointestinal	17	5	3	6	3
GU/Reproduction	3	1	1	0	1
Neurological	5	1	3	1	0
Dermatological (hives, cat bite)	2	1	0	0	1
Musculoskeletal	31	4	9	10	8
Increased Knee Pain	19	4	5	4	6
Other	6	1	1	0	4
TOTAL	88	18	25	23	22

this unique complex [13,14,16]. While the prominent mineral present in Aquamin is calcium (dosage = 80% Ca U.S RDA), its role in joint health is unclear. Magnesium however, was given at the daily dosage providing 14% (male) to 18% (female) U.S. RDA [12] and over the course of this study, this increased consumption of magnesium may have influenced OA symptoms by affecting the utilization of calcium or by potentially reducing inflammation around the affected joint. Both manganese and selenium were given at the daily dosage providing up to 16% and 4% of their RDA respectfully. Both of these trace minerals have been reported to reduce the appearance of osteoarthritic lesions and reduce the severity of symptoms in OA [14,16].

These pilot trial results suggest a potential treatment effect for Aquamin among subjects with moderate to severe OA and this preliminary finding warrants further study.

Competing interests

Marigot Ltd. provided funding for this clinical trial and the article processing charges to publish this work. Melanie Walsh is a paid employee of Marigot Ltd., the sponsor of this work and provided only medical writing support for this manuscript. The other authors declare that they have no other competing interests. Marigot Ltd approved the protocol and reviewed the manuscript before submission for publication and can be reached at: Strand Farm, Currabinny, Carrigaline, Co. Cork, IRELAND; Phone: 353-21-437-8727; Fax: 353-21-437-8588. Marigot Ltd did not participate in any of the data collection or statistical analyses reported herein.

Authors' contributions

JLF and MW co-authored the manuscript. JLF co-authored the protocol and directed the research team at MARC during the conduct of this trial. JLZ provided critical review of the manuscript, co-authored the protocol and provided medical monitoring services during the trial. MAK provided critical review of the manuscript and the protocol and provided statistical services for the design, execution and analysis of the data in this trial. All authors have read and approved the final manuscript.

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