MARINE MINERALS FOR HEALTH

Research & Publication Overview
Second Edition 2020

Contents

The Marigot Group Background

Marigot Ltd. was established in 1993 by Les Auchincloss, previously founder and major shareholder of Biocon Limited until the acquisition by the Quest Division of Unilever in 1989. Operating under a system that fosters an entrepreneurial approach, the core business involves the identification and development of naturally derived ingredients for the enhancement of Human, Animal and Plant Health.

During the last 24 years, Marigot Ltd has operated with a unique appreciation and mindful understanding of its raw material. The company has worked tirelessly with relevant stakeholders and external parties, ensuring that material is harvested responsibly and with maximum sensitivity to the environment. From this backdrop, Marigot has created and developed the market for marine multi-minerals, covering both the animal health and human food and nutrition sectors.

Today, its products are sold in over 40 countries through exclusively appointed distribution partners. A unique facet of Marigot's commitment to its raw materials has been its dedication to top-tier peer reviewed research. The company invests as much as 5-10% of sales turnover annually in research based programs, to further understand the efficacy of these natural mineral sources.

This approach coupled with processing technology, optimised inclusion systems and application development has allowed Aquamin products to be successfully included in a wide range of human foods and dietary supplement formulations. The company can count some of the world's leading blue-chip feed and food producers as its valued customers.

Marigot is a widely diversified group with a strong entrepreneurial spirit. The group has global capabilities in the areas of:

- Health & Nutrition
- Food Ingredients
- Brewing
- Mariculture
- Fermentation
- Horticulture

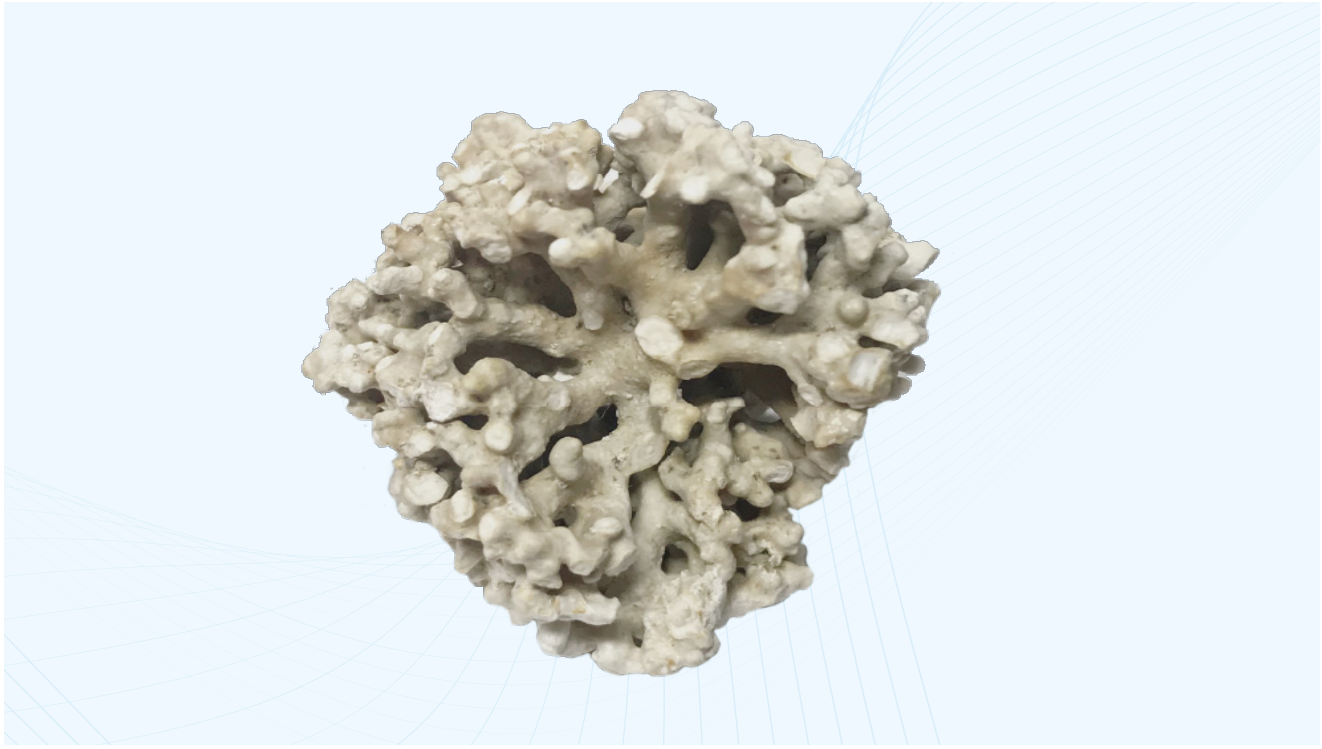


Fig 1: a fragment of harvested mature lithothamnion

About Aquamin Seaweed Mineral Complex

Aquamin is a natural, marine-sourced multi-mineral, which is derived from the cytoskeleton of the red algal *Lithothamnion* spp. Over the course of the aquatic plant's life, minerals are accumulated from the seawater, and stored as carbonate salts in the plant. 74 components have been identified in total.

Other than washing and milling, Aquamin is unaltered from the raw material and as such represents a natural multi-mineral material that is suitable for many food and supplement applications.

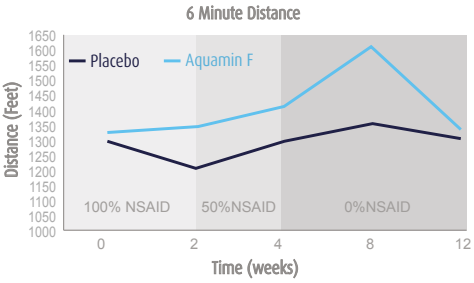
Although numerous anecdotal reports of health benefits associated with consumption of Aquamin existed, no objective research was carried out until Marigot Ltd. undertook to understand exactly how Aquamin could impact human health. Over 20 years, Marigot Ltd. have accumulated a large and growing body of research evaluating Aquamin in work spanning in vitro assays, numerous animal models and human trials. This portfolio of research has been conducted independently, and largely at academic institutions across the world,- by investigators that are renowned in their respective fields. All researchers are encouraged to publish their results on Aquamin in peer-reviewed scientific journals. As such, this research can be accessed by all and has withstood critique from peers in the relevant field(s).

Aquamin has been demonstrated to have superior bioavailability than other, commonly available calcium sources and has beneficial effects on bone, inflammation, specifically osteoarthritic conditions, digestive health, and cardiovascular health.

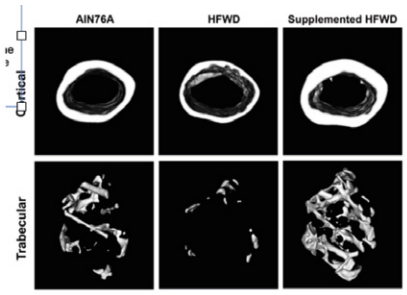
Key Dates

2008 Frestedt et al. (Nutr J) Aquamin shown to reduce symptoms of osteoarthritis.

2009 Fredstedt et al., (Nutr J) Aquamin significantly reduces NSAID intake due to improvement of symptoms of OA.



2010 Aslam et al. (Calcif Tissue Intl). Aquamin protects bone from the deleterious effects of a high fat western style diet and reducing polyp growth in mice.

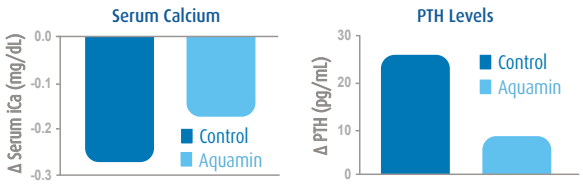


2011 Ryan et al. (Phytother Res) Aquamin reduces activity of the pro-inflammatory molecules, TNFα and IL-1β.
Barry et al., (Med Sci Sports Exerc) Aquamin protects bone during vigorous exercise (male cyclists).

2012 O'Gorman et al., (Phytother Res) Aquamin improves the growth, mineralisation and maturation of bone cells in-vitro.

2013 Aviello et al., (Phytother Res) Aquamin reduces inflammation and improves survival in an animal model of colitis.

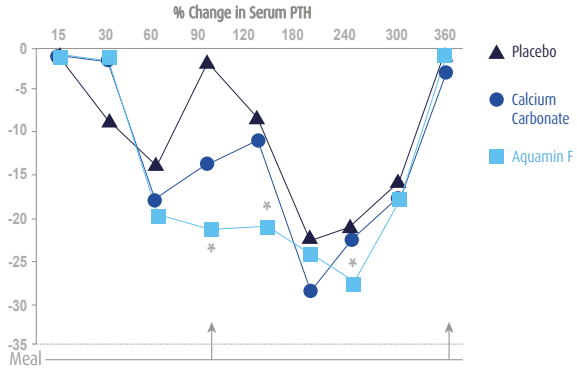
2014 Slevin et al., (J Nutr) Aquamin improves the bone health profile in post-menopausal women.
Widaa et al., (Phytother Res) Vit D improves the activity of Aquamin in bone cells in-vitro.
Shea et al, (Med Sci Sports Exerc) Aquamin protects bone during exercise (post-menopausal women).



2016 Marigot wins Ingredient of the year at the Nutraingredients Awards in the Healthy Ageing Category.

Cronin et al., 2016 (Br J Nutr) Aquamin improves cardiovascular health in post-menopausal women preventing an increase in bad (LDL) and total cholesterol.

2017 Zenk et al., (J Med Foods) Aquamin shows significantly greater bioactivity than CaCO3 in young adult females (Measured by PTH).



2019 Aslam et al., (Cancer Prevention Res) Aquamin improves the gut microbiota leading to a reduction in bile acids and a beneficial improvement in Short Chain Fatty Acids (SCFAs) in humans.

2020 Heffernan et al., (Comp Ther Med) Aquamin significantly reduced pain levels and NSAID intake in human subjects/patients with OA. (and to a greater extent than Glucosamine).
McClintock et al., (PLOS One) The most recent of a series of publications where patient colon cells were grown in-vitro showing the benefits of Aquamin in improving barrier impermeability of the Digestive tract, and increasing levels of protein required for organizing this barrier and transporting molecules across the barrier into the blood stream.

Bone Health Research

Osteoporosis is a major public health concern causing more than 8.9 million fractures annually worldwide, or one every 3 seconds, with post-menopausal women at particularly high risk. Scientific evidence has shown that dietary intake of calcium and other minerals is crucial to slow age-related bone loss.

To determine if the minerals in Aquamin might play a role in this important subject, the studies undertaken by Marigot in recent years have been comprehensive and have yielded impressive results on the benefits of Aquamin on bone health.

Our largest human dietary intervention study was carried out in conjunction with the University of Ulster, Ireland. Slevin et al.[1] investigated the effects of Aquamin alone and in combination with the prebiotic, short-chain, fructo-oligosaccharide (scFOS) on bone health, specifically bone mineral density (BMD) and bone turnover markers in 300 healthy, post-menopausal women over the course of 24 months.

There were 3 treatment groups. The first was a placebo group, in the second the women were given Aquamin only (2400mg/800 Ca/day) while in the third group, the women were given Aquamin in combination with scFOS (3.6g/day)). DEXA scans for BMD were taken at the beginning and end of the study while the bone breakdown marker (CTX) and the bone formation marker (osteocalcin) were measured at 0, 12 and 24 months.

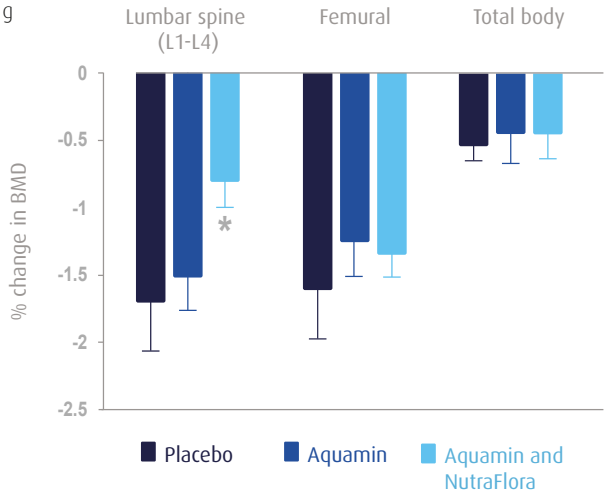


Fig 2: DEXA scans confirm bone protection in the lumbar spine in the combination group.

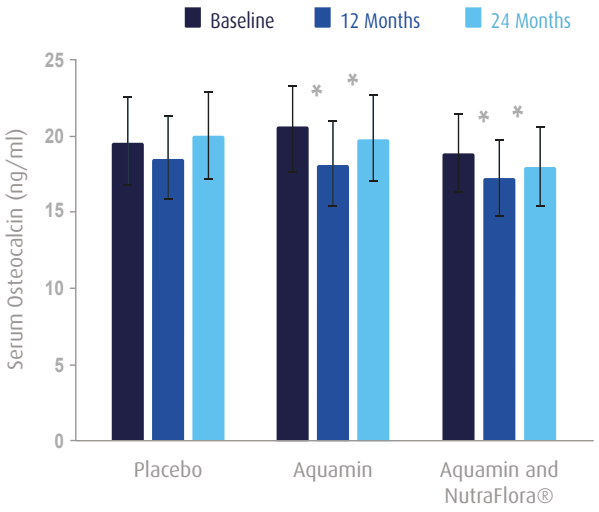


Fig 3: Significant changes were seen in the bone markers, osteocalcin and CTX, in both treatment groups.

The results above led us to ask questions about the bioavailability levels of Aquamin. To answer this we carried out a double-blind, randomised, cross-over study on young, healthy, adult females (Zenk et al., 2017)[2]. Twelve fasting female subjects received a single, oral dose of Aquamin (720mg Ca), or $CaCO_3$ (720mg) or placebo. Blood and urine samples were collected at baseline and over 12 hours to evaluate ionised and total calcium and parathyroid hormone (PTH) levels.

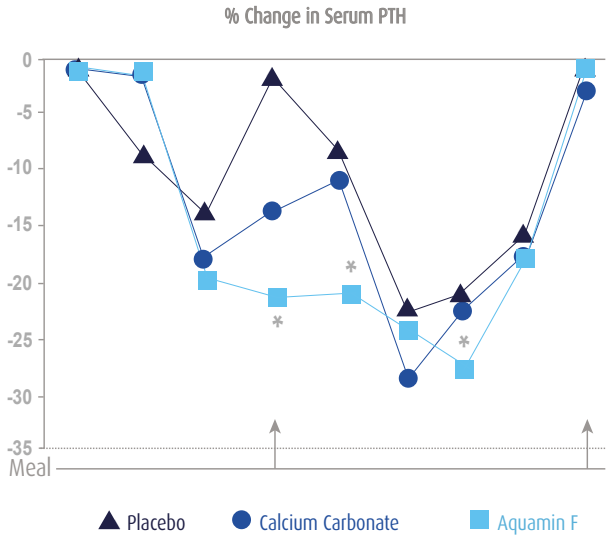


*Fig 4: Percent change in serum PTH levels over time. *Compared with placebo, the decrease in PTH concentration following Aquamin FTM treatment was significant at 90, 120, and 240 min (P= .0003, P = .017, and P = .030, respectively) while calcium carbonate treatment was significantly different from placebo treatmen only at 90 min (P = .026). Arrows indicate the timing of meals. PTH, parathyroid hormone. Color images available online at www.liebertpub.com/jmf*

The results showed that the subjects treated with Aquamin F demonstrated a prolonged and statistically significant suppression of serum PTH concentration compared to placebo at 90, 120, and 240 minutes while calcium carbonate showed an intermediate response. Furthermore, subjects treated with Aquamin F demonstrated significantly greater urinary clearance of calcium after 12 hours compared with placebo while the urinary clearance of $CaCO_3$ was not significantly different from placebo treatment. These results together show that Aquamin F demonstrates greater influence over these markers of calcium metabolism than inorganic $CaCO_3$.

We next investigated if Aquamin could protect bones during exercise. Exercise has long been recommended for both the prevention and treatment of low BMD. However, intense exercise has also been associated with a decrease in BMD under certain conditions, such as non-weight bearing sports, like cycling. In these circumstances, intense exercise can reduce serum calcium levels through perspiration, and increase parathyroid hormone (PTH) levels. These changes over time can reduce bone density. Our first exercise-related study (Barry et al.)[3] was carried out on 20 young, male, competitive cyclists in a double-blind, cross-over trial. The cyclists completed three 35 km time trials in the laboratory lasting on average 1 hour. They were given a different treatment for each trial, either 1000mg of Aquamin before the trial, 250mg of Aquamin every 15 minutes during the trial or placebo.

Exercise will normally induce an increase in PTH levels as calcium is lost through the skin as we perspire. Here, Aquamin before exercise significantly reduced the expected increase in PTH levels relative to placebo ($p<0.05$) and there was a similar trend for Aquamin delivered during exercise ($p = 0.07$). There were no significant changes in any other parameters measured.

	Placebo	Aquamin Before	Aquamin During
PTH (pg/mL)	74.0 ± 63.6	55.8 ± 67.2*	55.8 ± 67.2†
CTX (ng/mL)	0.24 ± 0.27	0.17± 0.21	0.17± 0.21
BAP (U/L)	1.4 ± 4.0	1.2 ± 3.5	1.2 ± 3.5
iCa (mg/dL)	-0.24 ± 0.20	-0.24 ± 0.16	-0.24 ± 0.16

Values are changes in response to exercise (mean ± SD)
*Different than placebo, * $p<0.05$, † $p=0.07$*

We next investigated whether these changes also occur in postmenopausal women (who are already at risk of osteoporosis) undergoing 1 hour of moderate exercise, and whether Aquamin supplementation before and during exercise can offset these changes (Shea et al.).[4]

We carried out 2 experiments using a randomized, double-blind, cross-over methodology. In the first experiment 10 women consumed a sports drink supplemented with 1000mg Calcium from Aquamin starting 1 hour before exercise (1 L total over 2 hours) or placebo. In the second experiment 23 women consumed a sports drink supplemented with 1000mg Calcium (Aquamin) starting 15 mins before exercise (1 L total over 1.25 hours) or placebo. Again the measurements included serum calcium, PTH and CTX. Increases in PTH and CTX (a bone breakdown marker) are undesirable as this indicates bone breakdown.

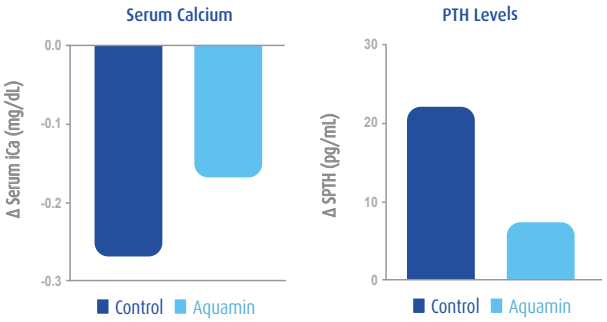


Fig 5a and Fig 5b: Shea et al. Changes in Serum Calcium and serum PTH are diminished when Aquamin is consumed (compared to control) in post-menopausal women undergoing a 60-minute brisk walking exercise test.

The results from the first experiment demonstrated that Aquamin prevented Calcium decrease (Fig 5a), and reduced PTH and CTX increases (Fig 5b) when consumption began 1 hour before exercise. The second experiment demonstrated that Aquamin reduced the Calcium loss but did not affect PTH and CTX increases when consumption began 15 minutes before exercise. Together these results indicate that Aquamin consumption 1 hour before and during exercise may prevent exercise-associated bone density reduction in post-menopausal women.

Typically, osteoporosis is thought of as a disease of post-menopausal women, but approximately 20% of cases and 30% of the osteoporosis-related bone fractures in Western societies occur in men. Regardless, our understanding of this disease is based on studies in females.

This next study examined bone structure and function in male mice maintained on either a control diet or a high-fat, Western-style diet (HFWD) for 18 months (Aslam et al., 2016)[5]. The HFWD as described previously is high in saturated fat and processed carbohydrates and is low in fibre, calcium & Vit D.

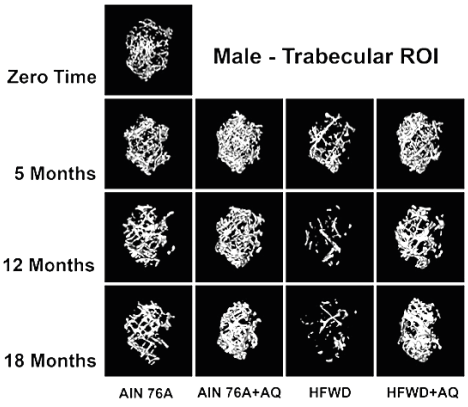


Fig 6 a

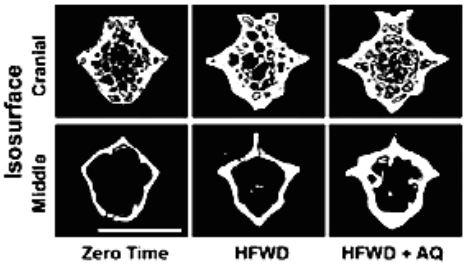


Fig 6 b

This study taken together with our other bone studies allow the conclusion that while female mice lose bone over time on both healthy and unhealthy diets (Fig 7), bone loss in male mice is, primarily, a consequence of the unhealthy HFWD (Fig 6 a). Moreover, the benefits of Aquamin supplementation in males were seen primarily in the HFWD, where bone loss was more severe over time. Similar results are seen in vertebral bone where Aquamin improves bone in HFWD-fed mice (Fig 6 b). Interestingly, strontium levels were increased several fold in the bones of Aquamin-supplemented mice (Fig 8). This is of particular interest as strontium is known to replace calcium in bone and preserve bone microarchitecture and bone strength. Indeed, strontium ranelate is employed as a medical treatment for osteoporosis.

The above results built on a previous publications also by Aslam et al., from 2013[6] and in 2010[7]. These studies also investigated the effects of Aquamin on bone structure and function and as one of the risk factors for bone heath is a poor diet, it also incorporated the HFWD. This diet was especially designed to mimic food consumption patterns of individuals in Western Society in mice.

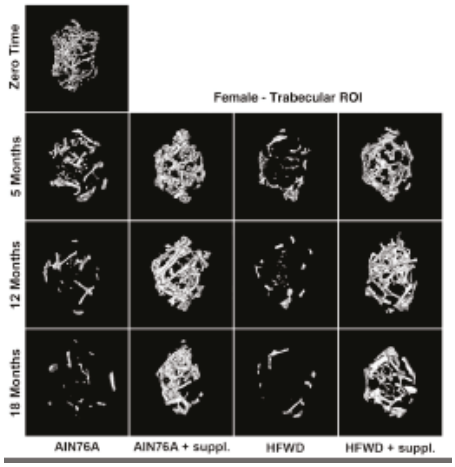


Fig 7

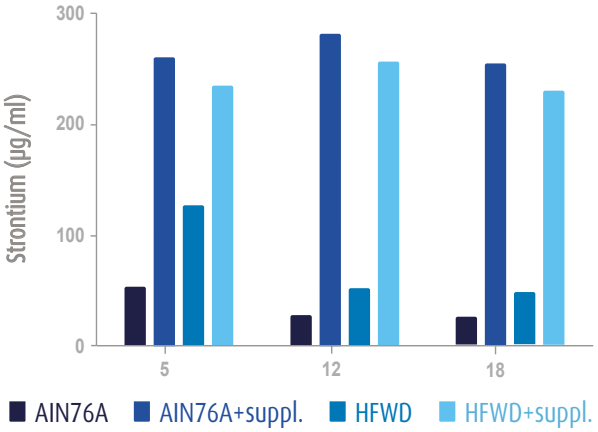


Fig 8

Joint Health, Osteoarthritis and Inflammation.

Osteoarthritis (OA) is the most common form of arthritis affecting millions of people worldwide. It is an inflammatory condition with symptoms that include pain, stiffness, reduced range of motion and functional disability 1. It has no cure and is managed primarily, particularly in the early stages, by Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) and Acetaminophen (also known as Paracetamol).

Marigot's most recent investigation (completed in 2020) into OA is also the most comprehensive.[8] For this study we compared Osean 74 (a Marigot product containing Aquamin F, sea-water derived Magnesium and pine bark) against Glucosamine on symptoms of pain and physical function in subjects with mild to moderate OA 3. Glucosamine is considered the most widely used nutraceutical for treatment of OA despite a lack of scientific consensus.

This recent study was a double-blind, randomised, cross-over trial where 30 subjects were assigned to either the glucosamine group or the Aquamin product for 12 weeks. This was followed by a 4-week wash out phase and 12 weeks of the alternate treatment. The Knee Injury and Osteoarthritis Outcome (KOOS) Score was used to assess pain and symptoms and analgesic use was recorded. Functional change was assessed by a Timed-up-and-Go (TuG) test and a six-minute walking distance.

The results for the Aquamin product were unequivocal – the pain of mild to moderate symptomatic OA was reduced for all participants (Fig 9 a). Indeed, this improvement in pain demonstrated a clinically important difference (or improvement) for both genders independently,

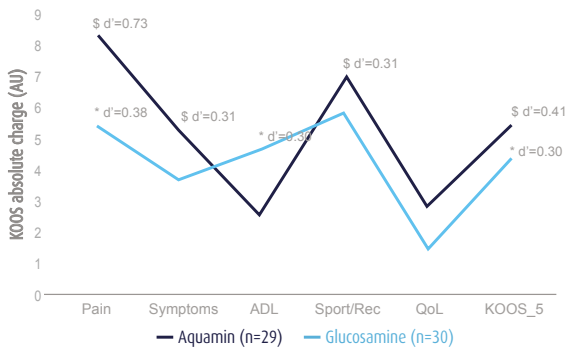


Fig 9 a: KOOS outcome score for all participants

and was found to be superior to Glucosamine. The results also proved that the use of analgesic medication was 72% lower and NSAID intake was 65% lower when participants were on the Aquamin product arm compared to Glucosamine (Fig 9 a). The results describing the reduction of pain medication is important as these medicines are known to have undesirable side-effects. Therefore, any reduction in their use, without an accompanying increase in pain is very beneficial.

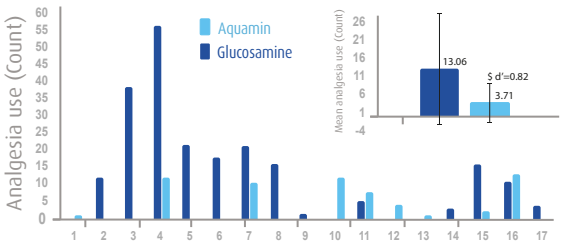


Fig 9 b: Individual participant analgesia use during the Glucosamine arm and the Aquamin product arm. Mean analgesia use for both products in the insert.

The above results consolidate all our previous OA studies. In 2008 the first Marigot publication on OA described how Aquamin improved the symptoms of OA reducing pain levels and stiffness while also increasing activity levels (as assessed by WOMAC scores) and walking distance.[9]

For this 12-week, randomised, blinded, parallel pilot study, 70 subjects with moderate to severe OA of the knee were recruited and divided into 4 groups (placebo, Aquamin, Glucosamine sulphate and lastly Aquamin and Glucosamine combined). The results were very clear, Aquamin showed significant improvements across all the WOMAC scores (Fig 10 a) and the 6-minute walking distance (Fig 10 b) and these improvements were significantly greater than those seen with Glucosamine.

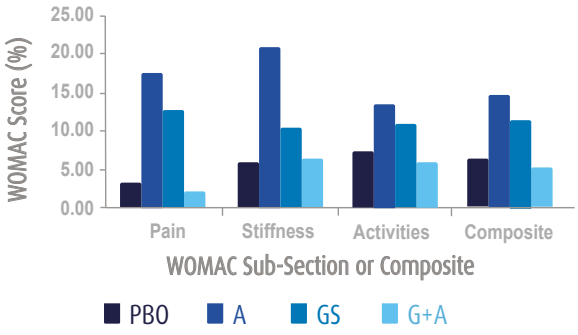


Fig 10 a Aquamin showed significant improvements for all WOMAC parameters.

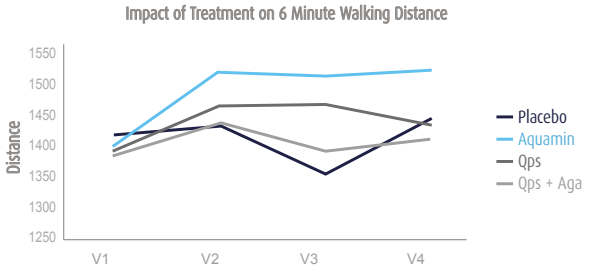


Fig 10 b This figure demonstrates the improvement in walking distance over the 12-week study. The most beneficial effects are seen with Aquamin (pink line).

The above study was quickly followed up by a second publication from the same group in 2009.[10] For this study, we investigated if it was possible to reduce NSAID usage with Aquamin. Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used to relieve the pain and discomfort of osteoarthritis but often have unwelcome side effects including digestive and heart health issues.

Again, we recruited subjects suffering from moderate to severe osteoarthritis for this blinded, randomised and parallel study where Aquamin was compared to a placebo. Subjects were required to reduce NSAID use by 50% after 2 weeks of treatment and to stop NSAID use after 4 weeks on the study. No NSAID use was allowed for the remaining 8 weeks but subjects could take acetaminophen as a rescue medication for pain.

The results showed that with a 50% reduction in NSAID use, the Aquamin group showed significantly increased range of motion scores and 6-minute walking distance (Fig 11) 6. The chronic pain associated with OA should never be underestimated. Sufferers of OA often experience pain daily, weekly, monthly for years, often decades on end. Any reduction in NSAID use through moderation of pain levels is of enormous benefit to OA sufferers.

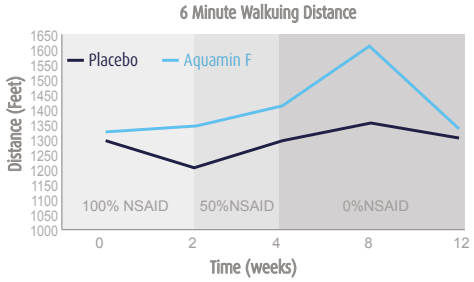
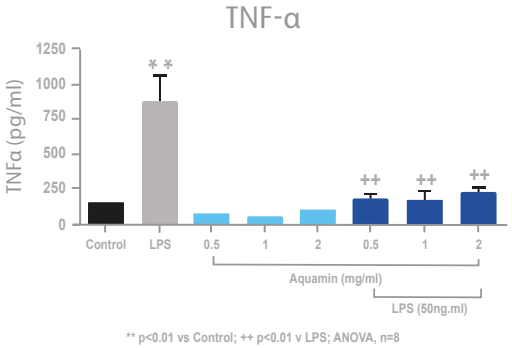


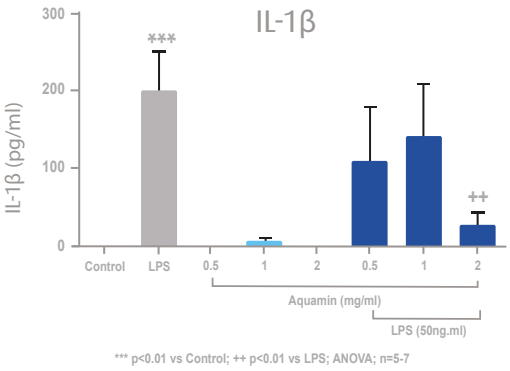
Fig 11: Subjects taking Aquamin treatment demonstrate significantly increased mobility as measured by 6MWD compared to placebo when NSAID use has been reduced by 50%.

To understand how Aquamin might mediate its observed anti-inflammatory effects, we investigated how cytokines are influenced by Aquamin.[11] Cytokines are a family of molecules that help to regulate the immune response and inflammation. This project aimed to elucidate the mechanisms by which Aquamin may alter these cytokine pathways as lack of regulation of these molecules, particularly Tumour Necrosis Factor (TNF)-α and Interleukin (IL)-1β can lead to inappropriate inflammatory responses.

Glial cells were used for this in-vitro study as they are particularly sensitive to inflammation. Inflammation was stimulated using bacterial Lipopolysaccharide (LPS) and the release of TNFα and IL-1β from the inflamed cells was measured.

The results were unambiguous. The control cells demonstrated no inflammation while LPS induced significant inflammation as measured by TNFα (Fig 12 a) and IL-1β (Fig 12 a) secretion. Aquamin itself at did not induce inflammation but significantly inhibited TNFα and IL-1β secretion from LPS treated cells.





Inflammation was determined by measuring TNFα (Fig 12 a) and IL-1β (Fig 12 b) secretion from cells stimulated in-vitro with LPS.

These results were reinforced by a pilot-scale human study (Murphy et al., 2014)[12] where 6-week treatment with Aquamin blended with green tea and pine bark (AquaPT) demonstrated a reduction in serum TNFα.

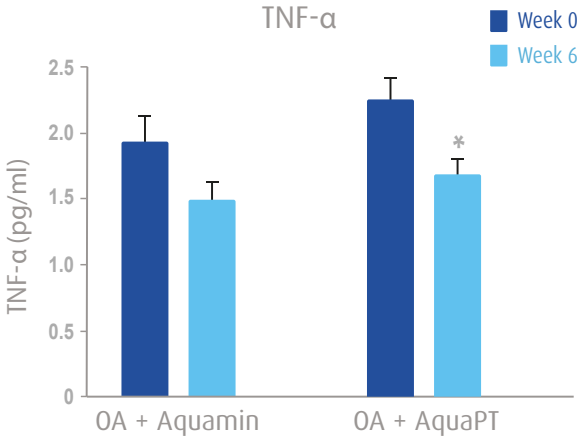


Fig 13: Effect of 6-week treatment with Aquamin or AquaPT on serum TNF-α levels in patients with O.

Another in-vitro study (O'Gorman et al., 2014)[13] investigated the effect of Aquamin on nuclear factor kappa B (NF-κB). NF-κB is another important and central regulator of the immune response and inflammation. Again, cells were cultured in-vitro and inflammation stimulated using bacteria LPS. Not only was partial inhibition of NF-kB recorded (Fig 14 a) but also inhibition of COX2 was evident (Fig 14 b) 9. NF-κB controls the expression of several key pro-inflammatory genes including cyclo-oxygenase 2 or COX2. COX2 is a well-established target for NSAIDs used to provide relief from inflammation and pain. A reduction in COX2 suggests that Aquamin may work through this same pathway.

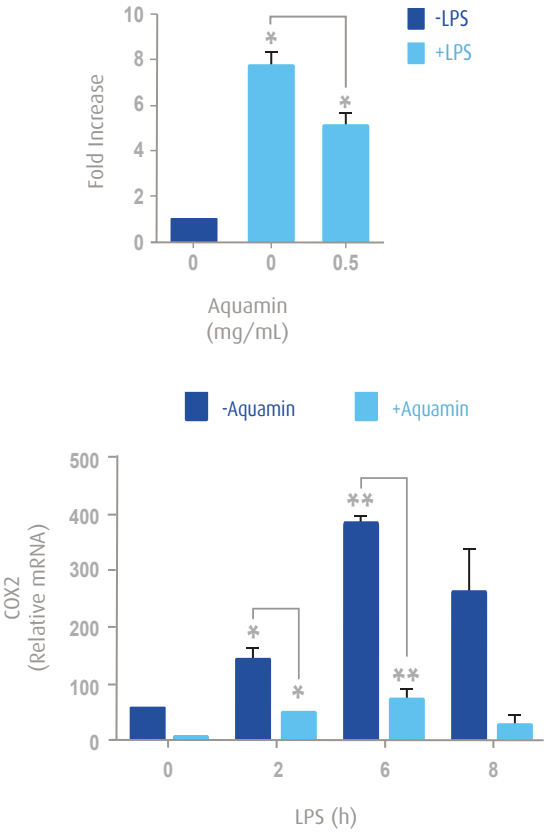


Fig 14 a Aquamin significantly inhibits NF-κB activity
Fig 14 b Aquamin inhibits LPS-induced COX2 expression

Gut Health, Microbiome Science & Aquamin as a Prebiotic

The impact of Aquamin on animal and human gut microbiota has been evaluated through comprehensive in vivo studies at independent, internationally-renowned, clinical centers of excellence for gastro-intestinal health and microbiome studies. Recent peer-reviewed studies, highlighted below, have demonstrated that Aquamin influences numerous parameters enhancing microbiome diversity.

The use of Aquamin as a carrier and synbiotic for probiotic bacteria allows these natural features of Aquamin to be exploited for human gut health and the health of the gut microbiota.

The effectiveness of Aquamin as an excellent buffering material has been extensively demonstrated through potentiometric titration analysis. This data provides support for the use of Aquamin as a protective carrier, for the transport of probiotic bacteria through the harsh conditions of the gastric phase of digestion. Hydrochloric acid produced by parietal cells lining the stomach is rapidly neutralized by the calcium carbonate superstructure keeping the probiotic cargo protected from the severe and lethal acidic conditions of the gastric phase of digestion.

Aquamin has been demonstrated to be an excellent carrier for probiotic bacteria (Fig 15), with excellent loading characteristics, stability, and viability.

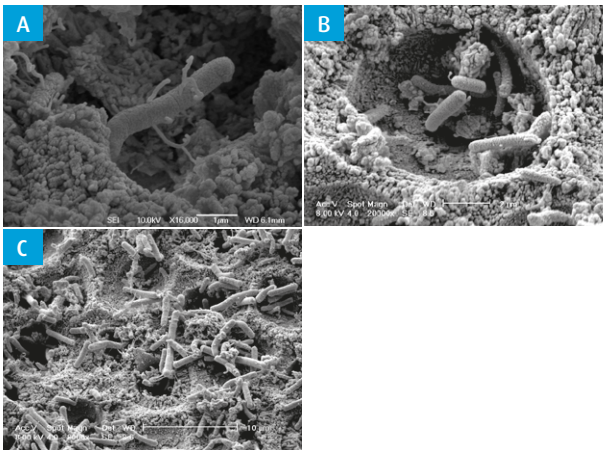


Fig 15: High resolution SEM of an Aquamin particle with Lactobacillus plantarum. A) shows the L. Plantarum cell secreting exopolysaccharides to attach to the Aquamin. B) and C) Show bacteria colonizing the pore spaces.

Marigot Ltd are a proud member of the Global Prebiotic Association (GPA). The purpose of the GPA is to raise awareness of the emerging and distinct health benefits of prebiotics.

The current international definition of a prebiotic was developed by a panel of experts in microbiology, nutrition, and clinical research convened by the International Scientific Association for Probiotics and Prebiotics (ISAPP) in 2016. This consensus definition for a prebiotic is "a substrate that is selectively utilized by host microorganisms conferring a health benefit".

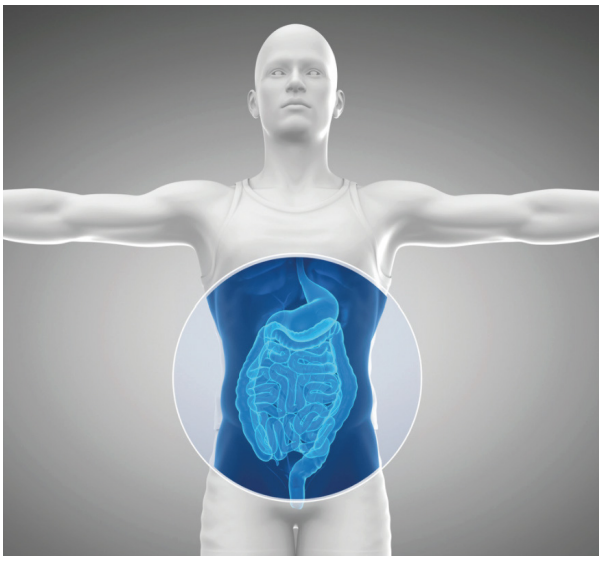
GLOBAL PREBIOTIC ASSOCIATION

Prebiotics are generally equated with dietary fibres. However only a subset of dietary fibres actually qualify as prebiotics. Now, according to this most recent scientific definition, prebiotics need not only be a form of dietary fibre. Rather, for a compound to be considered a prebiotic it must confer a beneficial physiological effect on the host by the selective utilization of a compound by gut bacteria.

Based on this definition and recent publications outlined here, Marigot Ltd and the GPA conclude that Aquamin is a prebiotic, with clear and measurable benefits to the gut.

Aquamin & Gut Inflammation

For the past decade, Marigot Ltd has worked closely with Professor James Varani and his colleagues at the University of Michigan Medical School. They tested the hypothesis that the absence or reduced intake of multi-minerals in a Western style diet, may contribute to diseases with a diet-associated factor. These investigations began in-vitro, with preliminary observations showing that cultured gut-lining (epithelial) cells showed improved differentiation (non-malignant effect and improved function) and proliferation (healthier growing cells) in the presence of Aquamin (Aslam et al., 2009).[14] These initial results demonstrated that the minerals in Aquamin helped maintain a healthy digestive barrier, which is necessary to prevent chronic inflammation in the gut. Follow-up studies investigating the role of Aquamin in the regulation of gastrointestinal inflammation in mice, subjected to a mouse version of the Western Style Diet, found a reduction in generalised inflammation in the gut, colonic polyp formation and fatty liver disease (Aslam et al., 2010).[15]



This finding was further supported in another trial, where mined limestone rock was compared against Aquamin. Despite each group of mice consuming the same amount of calcium, the mice receiving Aquamin were protected against GI inflammation and resultant polyp formation (Aslam et al., 2012).[16] An incidental finding from the study was significantly reduced liver mass formation in mice fed the Western Style Diet plus Aquamin versus the controls and limestone-derived calcium (Aslam et al., 2012, Biol Trace Elements).[17] Taken together, these results have prompted Marigot Ltd, alongside colleagues from the University of Michigan, to further investigate whether these anti-inflammatory effects in-vitro and in the digestive tract of mice can also be observed in humans. Initial results from this FDA-approved and regulated human trial describes how the results, show improvements and beneficial alterations in the biomarkers of differentiation (function) and proliferation (growth) in the presence of Aquamin, confirming that Aquamin helps maintain a healthy digestive barrier and reduce chronic inflammation in the human gut. A healthy, impermeable barrier is important as it prevents 'leakage' of undigested foods, bacteria and virus' into the bloodstream. The next step in this series of experiments is to investigate the effect of Aquamin in gut inflammatory conditions such as ulcerative colitis. These human studies are FDA-approved and regulated.

Previously, the beneficial effects of Aquamin were seen in a mouse model of colitis. Colitis is one of several chronic inflammatory disorders collectively known as inflammatory bowel disease (IBD). Current therapies target the inflammatory pathways with a view to resolving inflammation in the gut. Aquamin supplementation provided a significant reduction in mortality and disease activity along with significant reductions in several markers of inflammation including IL-1 β, TNFα and IL-2 (Fig 16) (Aviello et al., 2013).[18]

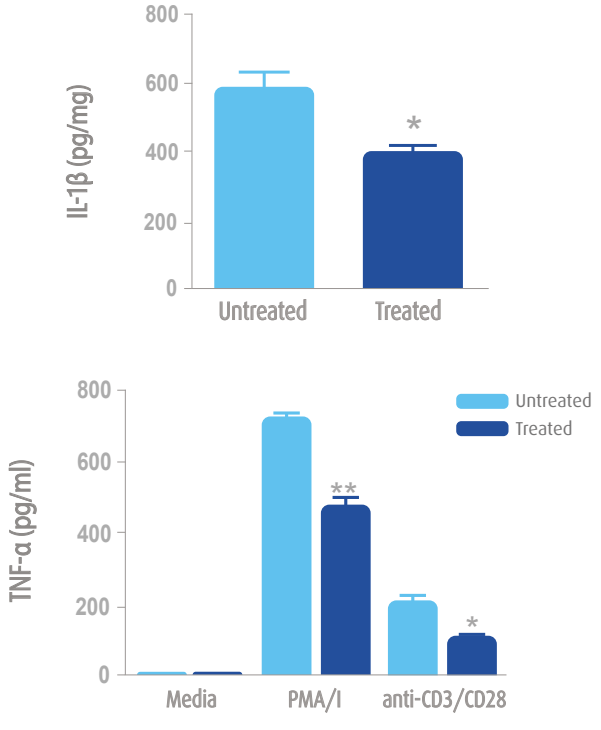


Fig 16: The impact of Aquamin (Treated) on 2 markers of active inflammation in Colitis. PMA/1and anti-CD3/CD28 represent different subsets of immune cells from the spleen.

Aquamin & Gut Microbial Population

A growing amount of scientific evidence suggests that colon microbiota of Irritable Bowel Syndrome (IBS) patients differ from that of healthy subjects. Alterations in several different microbial species, genera or groups have been reported but it is noteworthy that the results of different studies have to some extent been contradictory. An on-going in-vitro study assessing the impact of Aquamin on an IBS-derived, gut microbial population has led to some interesting results. In this study, the levels of gas production and individual short chain fatty acids (SCFAs) were measured in the absence of or with increasing doses of Aquamin. The three most abundant SCFAs are acetic, propionic, and butyric acids. In the colon simulation model used in the study, the total acid concentration was used to assess the overall fermentation activity while the relative abundance of individual acids indicated the respective activity of different fermentation pathways. Despite no observed change in overall gas production, a dose-dependent increase in total SCFA production was recorded, ranging from 9% to 17% when compared to the control. Acetate, associated with weight control and a healthy immune system, production was stimulated by between 7-9% and propionic acid by 17-44%. Interestingly, there were no significant changes in butyric acid levels at any time point or with any concentration of the dietary supplement. Lactic acid is the strongest of the common SCFAs produced by GI bacteria and its accumulation is considered a negative event for the lower intestinal tract. Inclusion of Aquamin resulted in decreased levels of lactic acid as well as a dose-dependent buffering action on colonic pH in comparison to the control treatment. These results indicate a substantial alteration in bacterial fermentation patterns and a more beneficial phenotype for relieving IBS symptoms which further investigated in dietary intervention studies.

The above results were reinforced in two more recent studies, one in animals and the other in humans. Crowley et al., 2018[19] demonstrated that the gut microbial diversity and species enrichment were significantly enhanced in adult rats when they were fed a blend of Aquamin and Aquamin Magnesium for six weeks. Furthermore, in our first FDA-approved human study, thirty healthy adult participants (10/group) were enrolled in a 90-day trial in which Aquamin (800mg Ca/day) was compared to calcium alone or placebo. Colon biopsies and stool specimens were obtained and analysed before and after the intervention and the changes to the gut microbiota were recorded and significant changes in microbiota were observed (Fig 17) (Aslam et al., 2019).[20]

This study also recorded a reduction in total bile acids and an increase in the level of the SCFAs, (Fig 18). SCFAs are produced when 'good' bacteria ferment indigestible foods. They are the main energy source of cells lining the colon making them crucial to gastrointestinal health. Taken together these results are considered highly beneficial for the gut. No significant changes in bile acids or SCFAs were seen with calcium alone or placebo.

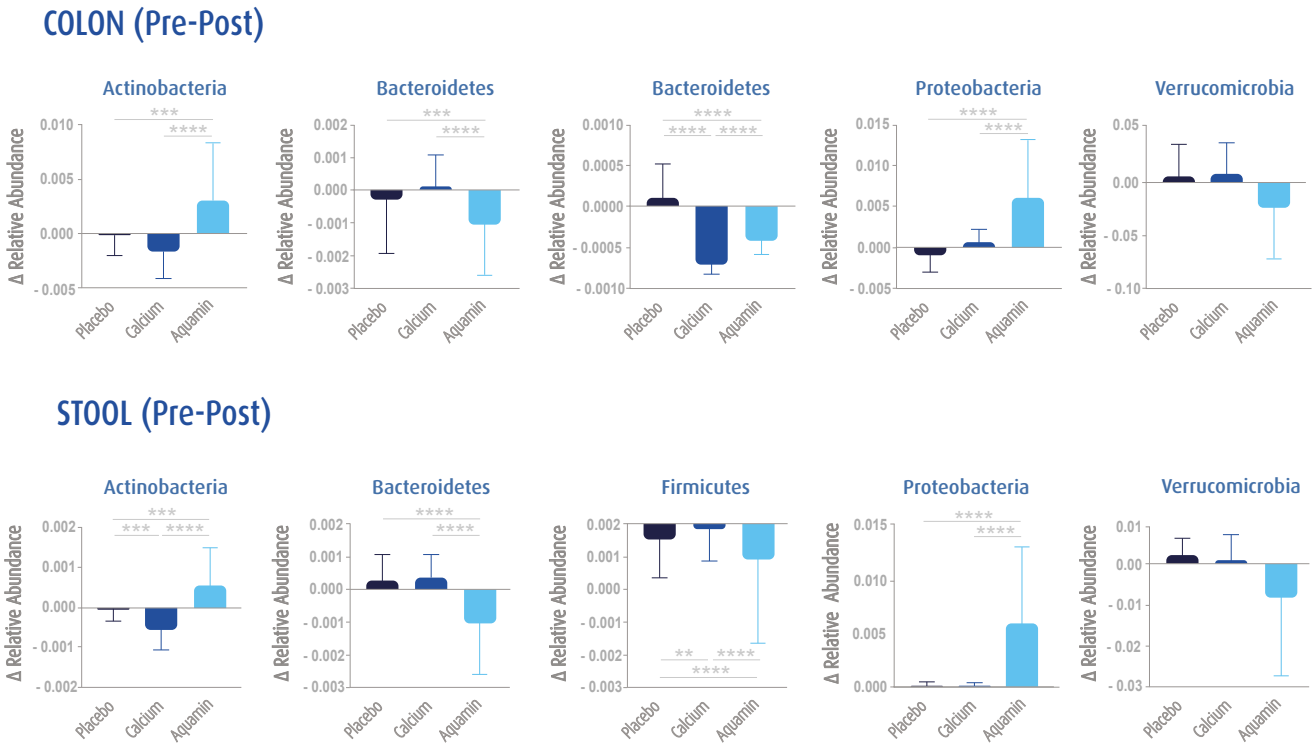


Fig 17: (A) The impact of Aquamin on SCFA profile and (B) the changes in gut microbial communities and diversity (relative abundance at phyla level) after 90 days of consumption.

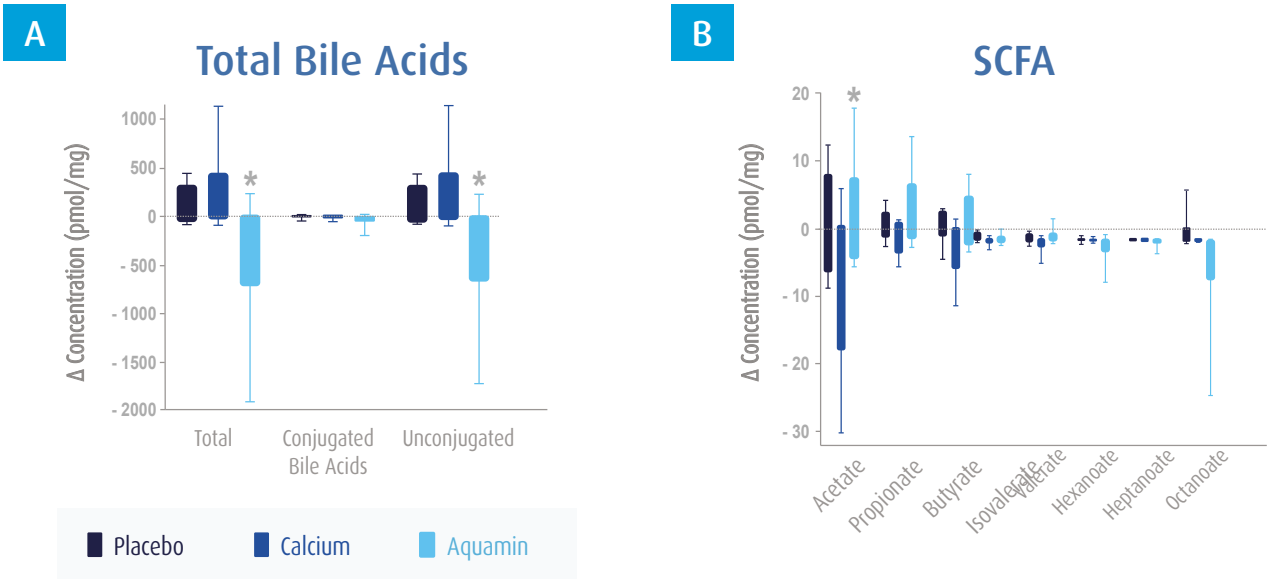


Fig 18: Decrease in bile acids and increase in SCFAs in stool specimens. Values shown represent concentration differences between pre-intervention and post-intervention samples. Asterisks represent statistical significance. (A) Total bile acids (sum of the total conjugated and total unconjugated bile acids) are shown along with conjugated and unconjugated forms. (B) SCFA. Acetate was significantly increased with Aquamin relative to calcium alone ($P < 0.0001$).

Aquamin, Polyp Formation and Gut Malignancy

The level of colon polyp prevention seen with Aquamin is clearly greater than previously evidenced for calcium alone. This reinforces the strong argument that minerals work more effectively when found together in their natural forms. Other minerals present in the marine algae, including copper, chromium, manganese, molybdenum, selenium and zinc, have all been shown to reduce tumour formation or suppress other types of tissue injury in gastro-intestinal tracts. It is thought that each of these elements may exert some level of protection against polyp formation by itself or they may well function synergistically with one another or with calcium. Marigot Ltd's digestive health studies prove that the minerals present in the marine algae, Aquamin, reduce colon polyp formation in both high-fat and low-fat diets (Fig 19). Calcium alone cannot explain the protective effects of the multi-mineral complex, and the many other additional minerals present are likely to contribute to colon health.

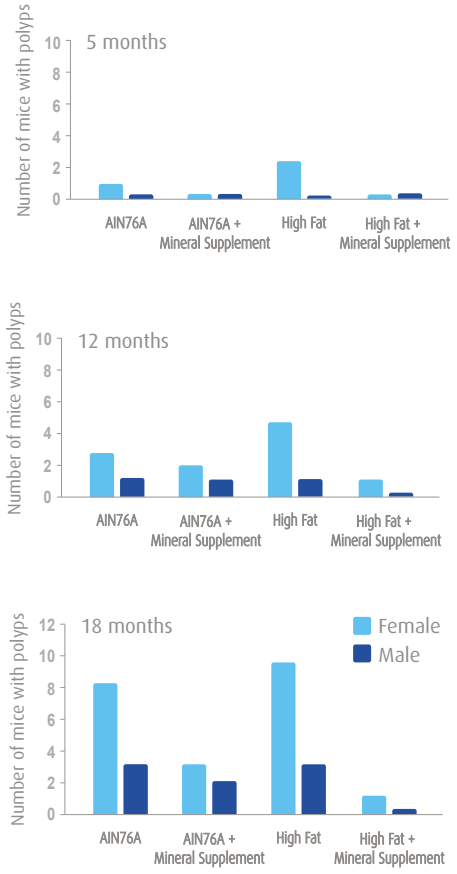


Fig 19: Colon polyp formation in mice fed Aquamin and either a normal or high-fat diet.

Dame et al, 2011[21] showed that treatment of human colon tissue in organ culture with Aquamin is sufficient to see immunohistochemical changes reflective of improved differentiation and to enhance the growth-control properties of calcium. In vitro work carried out using malignant cell lines focused on determining a potential mode of action involved in the reduction of malignant phenotypes. The calcium sensing receptor (CaSR) is a robust promoter of differentiation in colonic epithelial cells and functions as a tumor suppressor. Cancer cells that do not express CaSR (termed CaSR null) are highly malignant while acquisition of CaSR expression in these cells circumvents the malignant phenotype (Singh et al., 2015)[22]. CBS and HCT116 human colon carcinoma cell lines and the corresponding CaSR null cells isolated from these lines were used in this study to evaluate the effect of Aquamin versus calcium and vitamin D. All three components induced CaSR mRNA and protein expression and inhibited cellular proliferation in the parental and CaSR null cells (Fig 20). However, Aquamin was found to be the most potent in this regard.

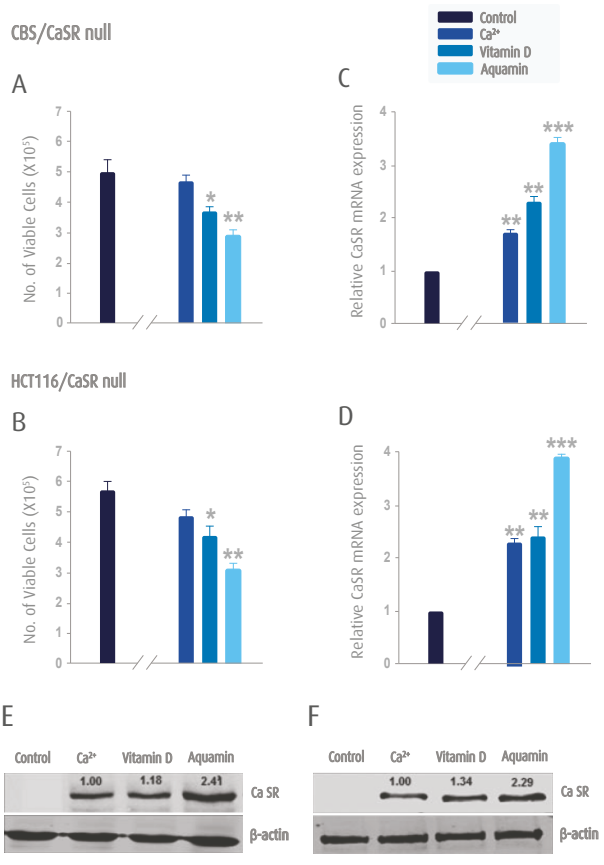


Fig 20: (A) and (B) Inhibition of growth of 2 human colon cancer cell lines by Aquamin. (C) and (D) Induction of Calcium receptor CaSR in 2 colon cancer cell lines which leads to a reduction in cell proliferation. (E) and (F) Western blot of CaSR expression in 2 colon cancer cell lines. Beta-actin used as standard.

Barrier Enhancement

Colonoid Studies

To explain the mechanism of action of Aquamin in the GI tract, a series of colonoid experiments were carried out. Colonoid culture is a well-developed technique where colon cells from patients are grown in the laboratory under ex-vivo conditions.

Aquamin was found to be more effective than calcium alone in increasing the proteins required for differentiation such as Occludin, E-cadherin, and CK20, indicating that the function of cells was improved (Fig 21) (McClintock et al., 2018).[23]

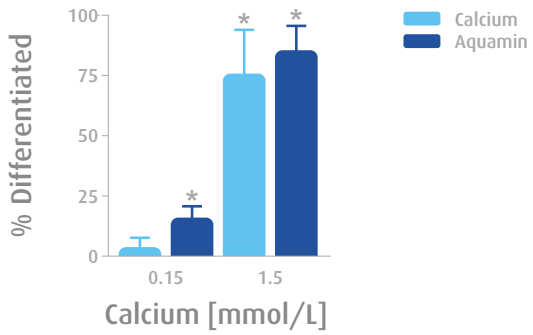


Fig 21: Colonoids maintained in calcium or Aquamin to provide either 0.15 mmol/L or 1.5 mmol/L calcium. Asterisks indicate statistical significance at P < 0.05.

Furthermore, the effects of Aquamin were much more pronounced in abnormal and inflamed tissue rather than normal control tissue.

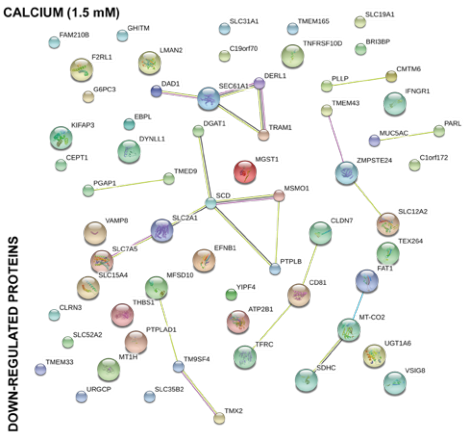
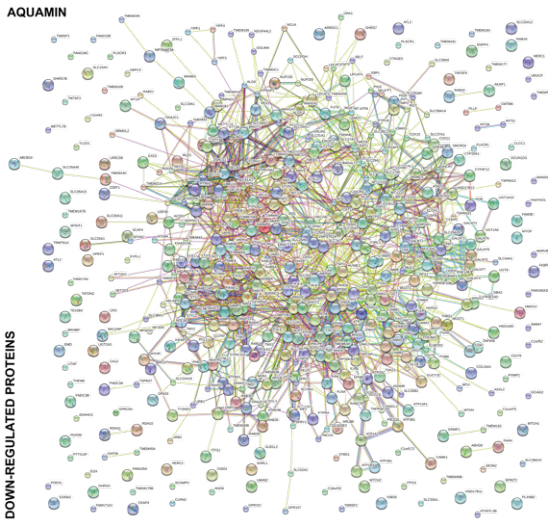


Fig 22 a: A representation of the proteins either increased or decreased in response to calcium or Aquamin as measured by proteomics.

Proteomics (the large-scale study of proteins) was also used to investigate the different proteins increased or decreased in response to calcium alone or Aquamin.



The results showed that Aquamin has a much greater effect on cell proteins than calcium alone (Fig 22 b) and re-enforced our earlier results.

- Aquamin greatly promotes cell-to cell adhesion
- Enhances barrier formation and integrity

The colonoid studies continued with Attili et al., 2019[24] where the proteins responsible for membrane impermeability were investigated. As per Fig 23, Aquamin serves to organise and systematically arrange the proteins most important to creating this vital impermeable membrane. The greatest improvements were seen with desmosomes (responsible for anchoring and connecting cells) and tight junctions (responsible for forming a seal between cells). Significantly more desmosomes are visible (Fig 24: white arrows) in the Aquamin treated cells as compared to calcium alone.

Together these studies provide a rationale for the use of a multi-mineral approach for a healthy gut.

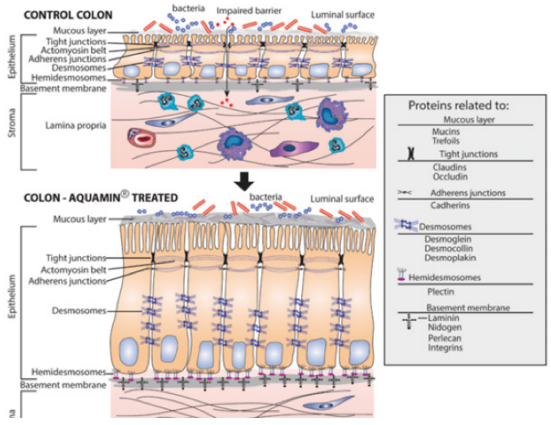


Fig 23: A schematic representation of the role of Aquamin in organising the gut epithelium.

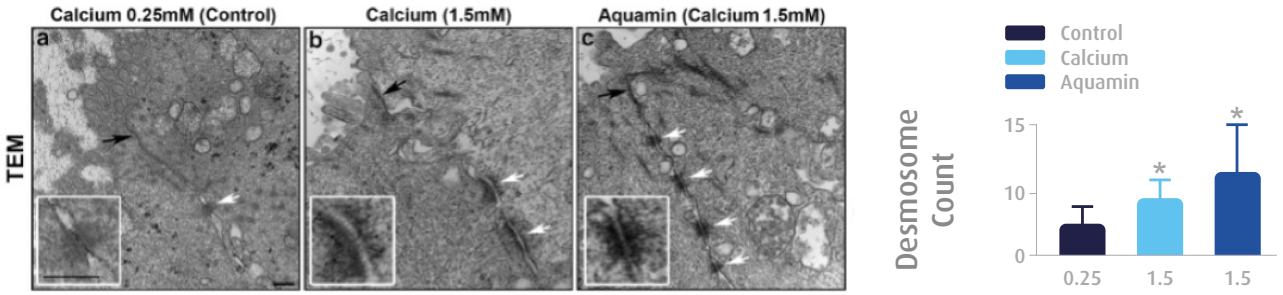


Fig 24: Ultrastructure by transmission electron microscopy. Under all conditions (a-c), tight junctions were evident below the epithelial layer on the luminal side (black arrows). Desmosomes were present in all conditions (white arrows) but a higher density of desmosomes were seen with Aquamin. Asterisks indicate statistical significance from control at p<0.05 level.

Marigot Ltd. have accumulated a large and continually growing body of gut health research evaluating Aquamin in work spanning in vitro assays, animal models and human trials. The results to date have demonstrated that the minerals in Aquamin may help maintain a healthy digestive barrier, which is necessary to prevent chronic inflammation in the gut.

Publication List

Bone Health

Publication	Title	Summary
1. Slevin et al., (2014) J Nutr 144(3) : 297-304	Supplementation with calcium and short-chain fructo-oligosaccharides affects markers of bone turnover but not bone mineral density in post-menopausal women.	Aquamin positively contributes to bone health as measured by DEXA and bone turnover markers in post- menopausal women.
2. Zenk et al., (2017) J Med Foods	Effect of calcium derived from Lithothamnion species on markers of calcium metabolism in pre-menopausal women.	Evidence of the difference in the bioactivity of Aquamin F vs CaCO3 in humans using PTH levels as marker.
3. Barry et al. (2011) Med Sci Sport Exerc 43(4):61723	Acute Calcium Ingestion Attenuates Exercise-Induced Disruption of Calcium Homeostasis	Aquamin taken before exercise protects from exercise-induced bone loss.
4. Shea et al., (2014) Med Sci Sports Exerc 46(10) : 2007 – 13	Calcium supplementation and PTH response to vigorous walking in postmenopausal women.	Aquamin consumption 1 hr before and during exercise may help prevent exercise-associated bone density reduction in post-menopausal women
5. Aslam et al., (2016) Bone Reports 5:141-149	Bone structure and function in male C57BL/6 mice: Effects of a high-fat western style diet with or without trace minerals.	This paper shows that the benefits of Aquamin in bone are not just limited to female mice but that significant benefits are seen in male mice too.
6. Aslam et al., (2013) Biol Trace Elem Res 156 (1-3) : 210-20	Preservation of bone structure and function by Lithothamnion species derived minerals	Aquamin inhibits bone mineral loss and improves bone strength and bone density. Strontium appears to play a central role.
7. Aslam et al. (2010) Calcif Tissue Intl. 86(4) : 313-24	A mineral-rich extract, Aquamin, from the red marine algae, Lithothamnion calcareum, preserves bone structure and function in female mice on a high fat diet.	Aquamin protects bone from the negative effects of a high fat diet
REVIEW: Heffernan et al., (2019). Nutrients 11(696)	The Role of Mineral and Trace Element Supplementation in Exercise and Athletic Performance: A systematic Review.	This review systematically considers the role of minerals and trace element supplementation in sports performance.
REVIEW: Aslam and Varani (2016). J Nut & Food Sci 6(3)	The Western-Style Diet, Calcium Deficiency, and Chronic Disease.	This comprehensive REVIEW discusses the Aquamin results from the Varani lab to date within the context of the western-style diet and chronic disease.
MG: Felice et al., (2018) Nutrients 10:912	Bioaccessibility and Bioavailability of a Marine-Derived Multimineral, Aquamin Magnesium	The bioaccessibility and bioavailability of Magnesium differs depending on the salt. Here Aquamin Mg is compared to MgCl2 and MgO.
Brennan et al., (2017) Calcified Tissue Intl	A natural, calcium-rich marine multi-mineral complex preserves bone structure, composition and strength in an ovariectomised rat model of osteoporosis	This paper clearly describes the benefits of Aquamin F in osteoporosis in this rat-model of human menopause.
Brennan et al., (2015) J Mechan Behav Biomed Mat 47:114-123	Incorporation of the natural marine multi-mineral dietary supplement Aquamin enhances osteogenesis and improves the mechanical properties of a collagen-based bone graft substitute	In vitro evidence that Aquamin can increase osteogenesis (i.e. production of new bone) thus improving bone formation, strength and mineralisation.

Publication	Title	Summary
Widaa et al., (2014) Phytotherapy Res; 28(5):678-84	The osteogenic potential of the marine-derived multi-mineral formula Aquamin is enhanced by the presence of Vitamin D	Vitamin D enhances the ability of Aquamin to promote mineralisation of bone cells.
O'Gorman et al., (2012) Phytotherapy Res 26 (3) : 375-80	The Marine-derived, Multi-mineral formula, Aquamin, Enhances Mineralisation of Osteoblast Cells In Vitro	Aquamin aids the mineralisation and maturation of bone cells.
Bae et al., (2011) Biol Trace Elem Res 144 : 992 – 1002	Magnesium supplementation through seaweed calcium extract rather than synthetic magnesium oxide improves femur bone mineral density and strength in ovariectomized rats	Aquamin is an effective calcium and magnesium source for improving bone health compared to synthetic calcium and magnesium supplementation.
Nielsen et al. (2010) J Equine Vet Sci 30(8):419-424	A marine mineral supplement alters markers of bone metabolism in yearling Arabian horses	Aquamin allows for rapid Breakdown and repair of bone in horses

Joint & Inflammation

Publication	Title	Summary
8. Heffernan et al., (2020) Complementary Therapies in Medicine	Mineral rich algae with pine bark improved pain, physical function and analgesic use in mild-knee joint osteoarthritis, compared to Glucosamine: A randomized controlled pilot trial	This publication compared Osean 74 to Glucosamine in subjects with mild to moderate osteoarthritis.
9. 16. Frestedt et al. (2008) Nutrition Journal 7 : 9	A natural mineral supplement provides relief from knee osteoarthritis symptoms: a randomized controlled pilot trial	Aquamin reduces the symptoms of osteoarthritis
10. Frestedt et al. (2009) Nutrition Journal 8:7	A natural seaweed derived mineral supplement (Aquamin F) for knee osteoarthritis: a randomised, placebo-controlled pilot study	NSAID usage can be reduced by Aquamin intake
11. Ryan et al. (2011) Phytotherapy Res 25(5): 765-7	Evidence that the marine-derived multi mineral, Aquamin, has anti-inflammatory effects on cortical glial-enriched cultures	Aquamin positively regulates the pro-inflammatory activity of TNFα and IL-1β
12. Murphy et al., (2014) J Nutr Health & Food Sci 2(3):1-3	The marine-derived, multi-mineral formula AquaPT, reduces TNFα levels in osteoarthritis patients	The anti-inflammatory action of Aquamin is enhanced in combination with pine bark & green tea
13. O'Gorman et al. (2012) Phytotherapy Res 26(3):630-32	Evidence that marine-derived, multi-mineral, Aquamin, inhibits the NFκB signalling pathway in vitro	Aquamin positively regulates the pro-inflammatory activity of NFκB
Cronin et al (2016) British Journal of Nutrition (115) 658 - 665	Effects of supplementation with a calcium-rich marine-derived multi-mineral supplement and short-chain fructooligosacharides on serum lipids in postmenopausal women	Long-term supplementation of Aquamin alone, or Aquamin+sc-FOS prevented increases in LDL and total cholesterol in post-menopausal women. No adverse effects on cardiovascular health were associated with supplementation
Hampton et al. (2015) J Am Assoc Lab Anim Sci 54(5): 487 - 96	Ulcerative demratitis in C57BL/6NCrl mice on low-fat and high-fat diets with and without a mineralized red algae supplement	Aquamin redcued the incidence and severity of ulcerative dermatitits in mice fed a high-fat western diet
O'Callaghan et al., (2013) J Medicinal Foods 16(10):920-6	Antioxidant and pro-apoptotic effects of marine-derived, multi-mineral Aquamin supplemented with pine bark extract, Enzogenol and green-tea extract, Sunphenon.	The antioxidant and pro-apoptotic effects of Aquamin are enhanced in combination with pine bark and green tea.

Digestive Health

Publication	Title	Summary
14. Aslam et al. (2009) Cancer Letters 283 (2): 186-92	Growth-inhibitory effects of a mineralised extract from the red marine algae, Lithothamnion calcareum, on Ca2+ -sensitive and Ca2- -resistant human colon carcinoma cells	Growth-inhibitory effects of a mineralised extract from the red marine algae, Lithothamnion calcareum, on Ca2+ -sensitive and Ca2- -resistant human colon carcinoma cells
15. Aslam et al. (2010) Integrative Cancer Therapies 9 (1): 93-9	A mineral-rich red algae extract inhibits polyp formation and inflammation in the gastrointestinal tract of mice on a high-fat diet	Aquamin protects the digestive system from inflammation and other negative effects of a high fat diet
16. Aslam et al., (2012) Nutrition in Cancer 64 (7), 1020-8	A Multi Mineral Natural Product Inhibits Liver Tumor Production in C57/BL6 Mice	Aquamin protects from polyp formation in the colon resulting from a high fat diet
17. Aslam et al. (2012) Biol Trace Elements Res 147: 267-74	A Multi Mineral Rich Natural Product from red marine algae reduces Colon Polyp Formation in in C57/BL6 Mice	Aquamin protects from liver disease from a high fat diet
18. Aviello et al., (2013) Phytotherapy Res. 28(2):300-4	A mineral extract from Red Algae Ameliorates Chronic Spontaneous Colitis in IL-10 Deficient Mice in a Mouse Strain Dependent Manner.	Aquamin significantly improves the symptoms of colitis in an animal model
19. Crowley et al., (2018) Marine Drugs	Dietary Supplementation with a Magnesium-Rich Marine Mineral Blend Enhances the Diversity of Gastrointestinal Microbiota	A 50:50 blend of Aquamin F and Aquamin Mg enhances the diversity of the bacteria in the gut of rats.
20. Aslam et al., (2019) Cancer Prevention Research	A Calcium-Rich Multi-Mineral Intervention to Modulate Colonic Microbial Communities and Metabolomic Profiles in Humans: Results from A 90-Day Trial	This study investigated if the benefits of Aquamin were mediated through effects on the gut microbial population and changes in microbial metabolic activity by measuring the colonic microbial community and metabolomic profile.
21. Dame et al. (2011) In Vitro Cell Dev. Biol. - Animal 47:32-38	Human colon tissue in organ culture: calcium and multi-mineral-induced mucosal differentiation	The multi-mineral Aquamin out-performs calcium in regulating cell growth in-vitro
22. Singh et al., (2015) Mol Carcin 54(7):543-53	Induction of calcium sensing receptor in human colon cancer cells by calcium, vitamin D and Aquamin: promotion of a more differentiated, less malignant and indolent phenotype.	Aquamin is much more effective than calcium alone in regulating the very important calcium sensing receptor.
23. McClintock et al., (2018) Cancer Prevention Res 2018	Calcium-Induced Differentiation of Human Colon Adenomas in Colonoid Culture: Calcium Alone versus Calcium with Additional Trace Elements	Aquamin F is significantly more beneficial than Calcium Carbonate in promoting normal growth in human colon adenomas ex-vivo.
24. Attili et al., (2019) PLOS ONE	Calcium-induced differentiation in normal human colonoid cultures: Cell-cell / cell-matrix adhesion, barrier formation and tissue integrity	This is a very interesting publication as it shows that there is no significant difference between calcium sources in normal epithelial tissue in colonoid culture
McClintock et al., (2020) PLOS ONE	Differentiation of human colon tissue in culture: Effects of calcium on trans-epithelial electrical resistance and tissue cohesive properties.	An intact colonic barrier is necessary for gastrointestinal health. This study investigates if Aquamin improved barrier function and barrier integrity more than calcium alone.