

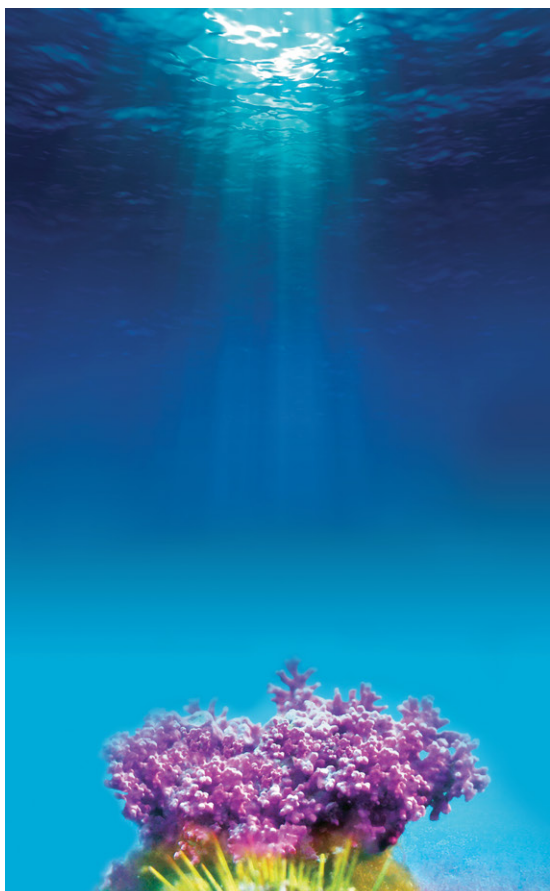


**GUT HEALTH &
MICROBIOME**

Enhanced
Immunity
Naturally

**MARINE
MINERALS
FOR HEALTH**

Introduction



Aquamin is a unique, natural offering for the enhancement of gut health. It contains significant amounts of calcium and magnesium as well as 72 additional trace minerals complexed together in a structure engineered by the cell wall of the seaweed *Lithothamnion* species.

Aquamin is used extensively in the fortification of food and nutraceutical applications and is supplied in several grades suitable for a wide range of formulations.

Aquamin has been the subject of 45 peer reviewed publications over the last 15 years. These support its unique health promoting properties, enhancing gut health by restoring a balanced immune response, promoting the differentiation of colonic cells and providing a balanced gut microflora.

The development of consumer demand for Aquamin was pioneered by Marigot Ltd. Marigot create awareness and provide innovative research data supporting the inclusion of the 74 marine minerals beneficial for animal and human nutrition in their diet. During this time, extensive human studies ensued to fully evaluate the clinical attributes present within the material, including bioavailability and bioactivity. Aquamin contains a unique trace mineral profile gained from its formation in a marine environment. The elements contained are at trace quantities and are insignificant alone, but within a multi-mineral matrix they work synergistically providing unique and robust bioactivities.

Harvesting

Aquamin is harvested under license in the cool, clean, and pristine waters off the coast of Iceland, in an area untouched by international shipping and industry. In this region, the warm waters of the gulf stream meet the cold, clean waters of the artic providing the perfect

conditions for deposits of *Lithothamnion* to grow. Marigot meet and exceed the highest international standards to ensure that the material is harvested in a responsible manner and that this precious resource is protected for future generations. An extensive quality control process ensures traceability, best quality, and consistency for each batch of Aquamin.



Fig 1: Beautiful Bildudalur, the home of Marigot Ltd, Iceland.

About Aquamin

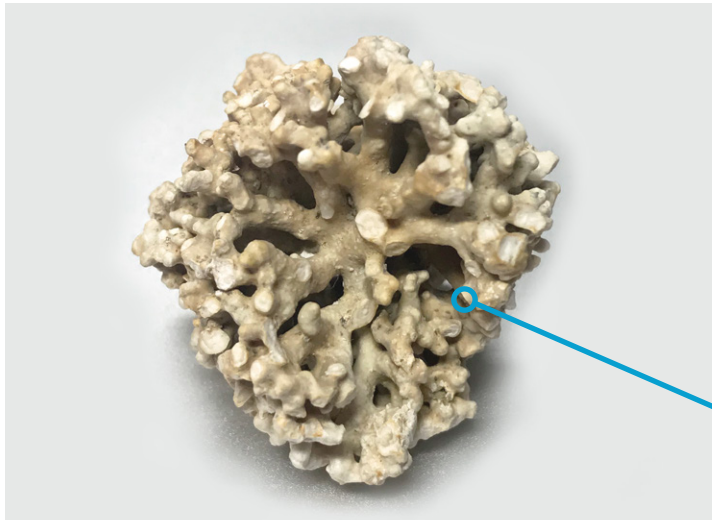
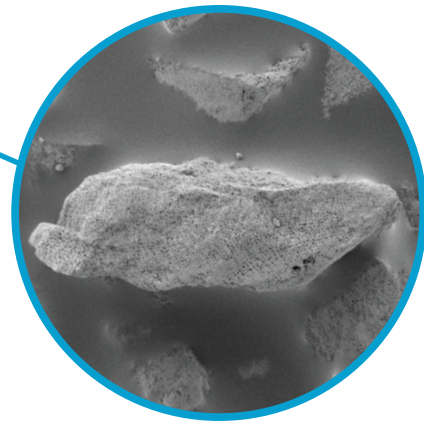
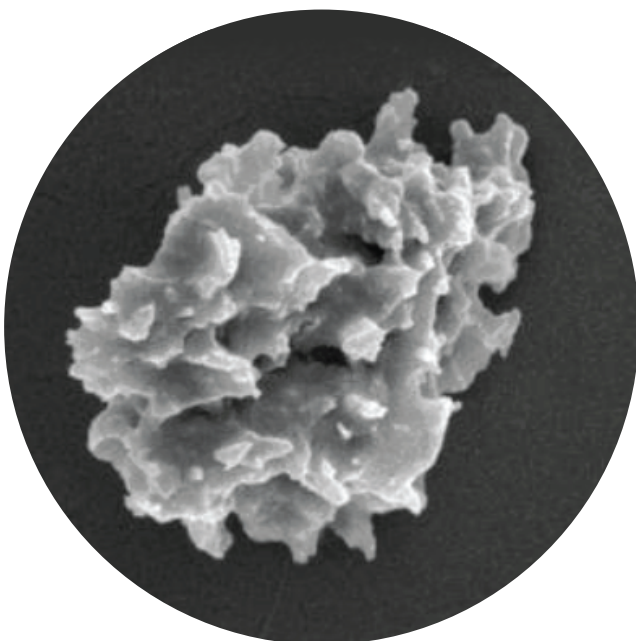


Fig 2: left, a fragment of harvested mature lithothamnion and right, a Scanning Electron Micrograph (SEM) of ground material that reveals the porous, micro-structure.



Aquamin is a marine-plant, multi-mineral complex, derived from the cytoskeleton of the red seaweed *Lithothamnion* sp. It is sustainably harvested from the pristine waters off the northern coast of Iceland. Over the course of the seaweed's life, minerals are accumulated from the seawater, and stored as carbonate salts in the plants cell wall. Calcium and magnesium represent one third and over 2%, respectively of the over 70 minerals components in the total dry mass of Aquamin. Aquamin has an intricate and unique 3-dimensional structure molded by the cell wall of *Lithothamnion* sp.

“ Aquamin differs elementally and structurally from mined minerals ”



A unique feature of Aquamin, in the context of the gut microbiota is its porosity, with each gram of Aquamin having a surface area of 8.9m²/g. This porosity is a result of the engineering of the *Lithothamnion* species bio-mineralisation process when the seaweed was growing in the ocean before it completed its lifecycle. This unique feature of the *Lithothamnion* sp. enables it to synergistically live with bacteria. The bacteria provide nutrients to the seaweed's cells while the seaweed provides protection from currents, a large solid surface area for colonization, essential minerals and favourable pH conditions (due to calcium carbonate backbone) for the bacteria to metabolise and thrive in this environment.

Fig 3: Further magnification of ground Aquamin particle by SEM

Gut Health

In today's fast-paced world highly processed, convenience foods have become a staple in our diets. This increasingly common and unhealthy way of eating has been dubbed "The Western Style Diet".

This diet is high in saturated fats, processed carbohydrates, sugar and is low in fresh fruits and vegetables, whole grains, and seafood. Additionally, the intake of many essential minerals is significantly lower than is recommended for good health. The diet is associated with a range of diseases including obesity, high blood pressure, high cholesterol, heart disease, type II diabetes, colorectal cancer, liver cancer, and osteoporosis, as well as inflammation and disorders of the digestive system.



Aquamin gut health benefits:

- Anti-inflammatory activity in the GI tract
- Beneficial changes to gut microbiota and short chain fatty acids (SCFAs)
- A reduction in the levels of bile acids and lactate production
- Maintenance of the impermeable membrane, healthier stomach wall
- Enhanced barrier structure and function in the digestive tract preventing 'leakage' of undigested foods, bacteria, toxins, and viruses into the blood stream
- Improvement of the symptoms of colitis
- Protection from liver damage while on a high fat Western diet (animal studies)
- Prevention of polyp formation on a high fat Western diet (animal studies)

Microbiome & Science

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, demonstrating selective utilization by the micro-organisms of the gut, allowing Aquamin to be considered as a mineral prebiotic.

Marigot Ltd. is a member of the Global Prebiotic Association (GPA). The GPA raises awareness of the emerging and distinct health benefits of prebiotics

GLOBAL

PREBIOTIC
 ASSOCIATION

Aquamin & Immunity - Beyond Prebiotic Activity

“A prebiotic is a substrate that is selectively utilized by host microorganisms conferring a health benefit”.¹ The benchmarking of Aquamin versus other prebiotic ingredients, as shown in Fig 4, highlights its robustness and efficacy in impacting numerous elements of gut health and the microbiome making it the ingredient of choice for natural immunity enhancement.

Aquamin enhances the diversity of the bacteria and beneficial changes in gut microbiota, increasing total short chain fatty acids (SCFAs), where acetate stimulation is associated with a support of healthier immune system.^{2,3,4}

Aquamin beneficially regulates the inflammatory mediators NF- κ B, TNF α , IL-1 β : & IL-4 central regulators of the immune response.^{5,6,7} Given the links between Aquamin and TNF α , IL1 β , NF κ B future research plans to solidify proposed link between Aquamin and regulation of the immune system.

Magnesium, present in Aquamin is also known to play an important role in the regulation of immune cell development and homeostasis, contributing to a healthier immune system. Magnesium supplementation may protect organs and tissues from damage through multiple mechanisms of action, including anti-inflammation, anti-oxidation and immune regulation⁸.

Marigot has not investigated yet the relationship between bone health and immunity directly. However, we have several indicators that Aquamin may play an important role in both areas of research and potentially in the developing field of osteoimmunology, investigating the complex interplay between bone health, general health, and immunity.⁹

Vitamin D is also known to play a central role in the correct functioning of the immune system as well as in osteogenesis. In 2014 Widaa et al., demonstrated significant synergy between Aquamin and vitamin D in a bone cell model¹⁰. The results showed enhanced bone growth or osteogenesis as measured by alkaline phosphatase (BAP) activity and a more uniform mineralisation pattern across osteoblast cells as measured by Alizarin Red staining. Alizarin Red not only stains for calcium but also magnesium, manganese, barium, strontium, and iron. These minerals are all found in Aquamin and are all important for bone formation. Further research is needed to investigate Aquamin synergy with D vitamin for immunity.



The prebiotic activity of Aquamin versus other prebiotic ingredients

PREBIOTIC Feature	Inulin	scFOS*	GOS*	AQUAMIN
Fiber Source	✓	✓	✓	X
Benefits Bacterial Energy	✓	✓	✓	X
Anti-Inflammatory Properties	X	X	✓	✓
Positive for SCFA production	X	✓	✓	✓
Dairy Free	✓	✓	X	✓
Boost Immune Function	✓	✓	✓	✓
Delivers Bifidogenic Effect	✓	✓	✓	✓
Negative Gastro Issues	✓	✓	X	X
Low Dose Effective	X	X	✓	✓
Barrier Enhancement	X	X	X	✓
Optimal Mineral Bioavailability	X	X	X	✓
Probiotic carrier functionality	X	X	X	✓
Gastric protection for probiotics	X	X	X	✓

Fig 4: The prebiotic activity of Aquamin versus other prebiotic ingredients.
 *scFOS - short chain fructo-oligosaccharides and GOS - Galactooligosaccharides.

Aquamin in a simulated digestion

The high-resolution SEM (Scanning Electron Micrograph) images in Fig 5 represent:

- A** The early stage of digestion where the Aquamin particle has not been significantly degraded.
- B** The latter stages of digestion where the calcium carbonate superstructure has been degraded during transit through the gastro-intestinal tract exposing greater surface area.

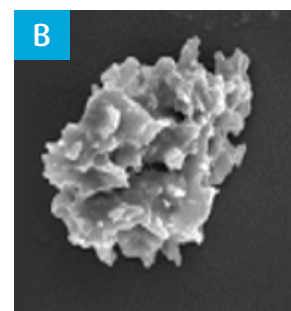
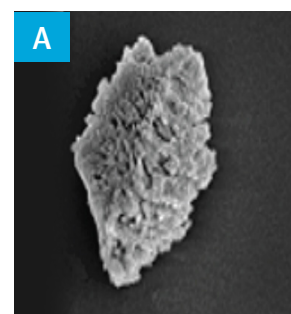


Fig 5: SEM images of Aquamin in the early (A) and late (B) stages of digestion.

Aquamin & Probiotics

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 6), with excellent loading characteristics, stability, and viability.

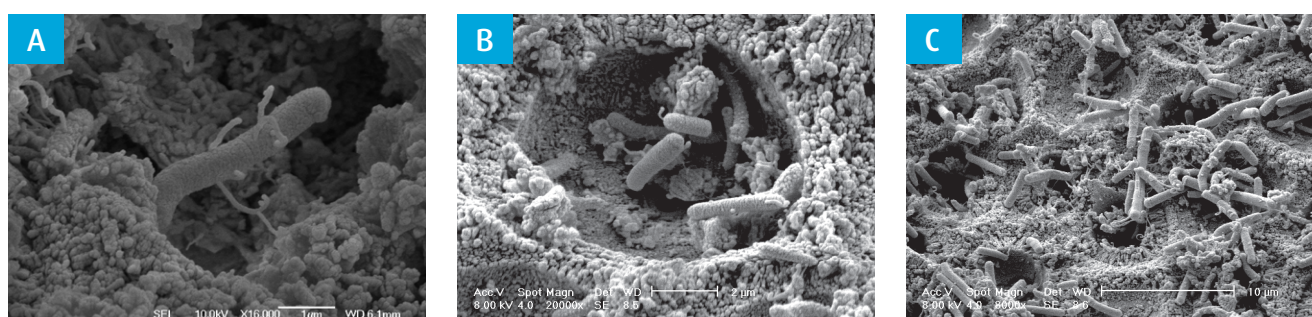


Fig 6: High resolution SEM of an Aquamin particle with *Lactobacillus plantarum*. A) shows the *L. Plantarum* cell secreting exopolysaccharides to attach to the Aquamin. B) and C) Show bacteria colonizing the pore spaces.

Aquamin and Gut Inflammation



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 formation¹³. An incidental finding from the study was

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 Aquamin¹¹. 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 disease¹².

significantly reduced liver mass formation in mice fed the Western Style Diet plus Aquamin versus the controls and limestone-derived calcium¹⁴. 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 will also be 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 , TNF α and IL-2 (Fig 7)¹⁵.

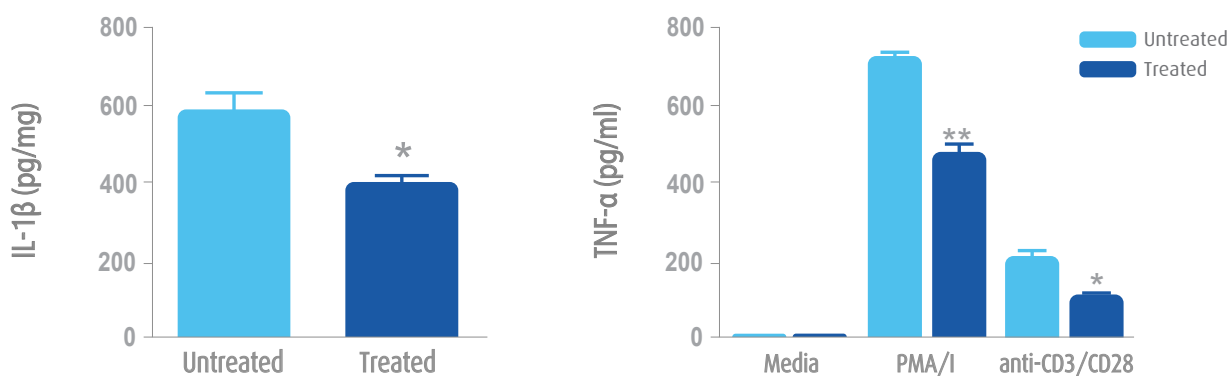


Fig 7: The impact of Aquamin (Treated) on 2 markers of active inflammation in Colitis. PMA/1 and anti-CD3/CD28 represent different subsets of immune cells from the spleen.

Aquamin and Gut Microbial Population

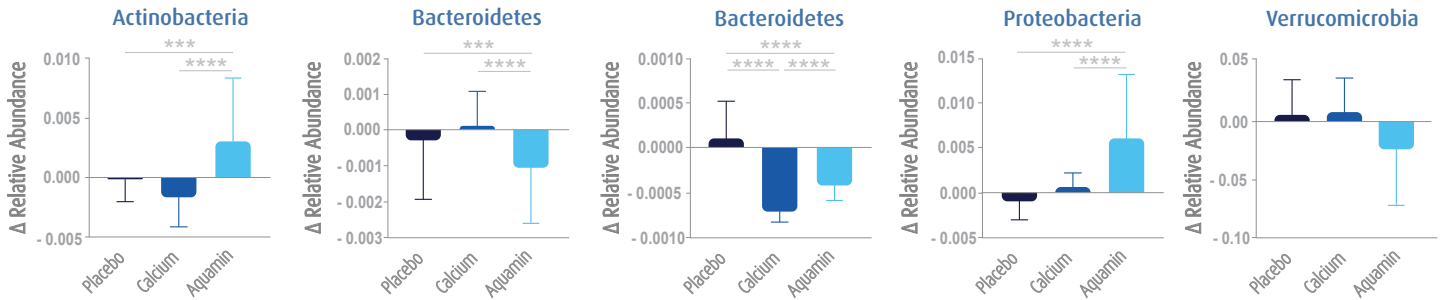
A growing amount of scientific evidence suggests that colon microbiota of Irritable Bowel Syndrome (IBS) patients differ from that of healthy subjects¹⁶. Alterations in several different microbial species, genera or groups have been reported but it is noteworthy that the results of different studies 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., 2018² 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 8)³.

COLON (Pre-Post)



STOOL (Pre-Post)

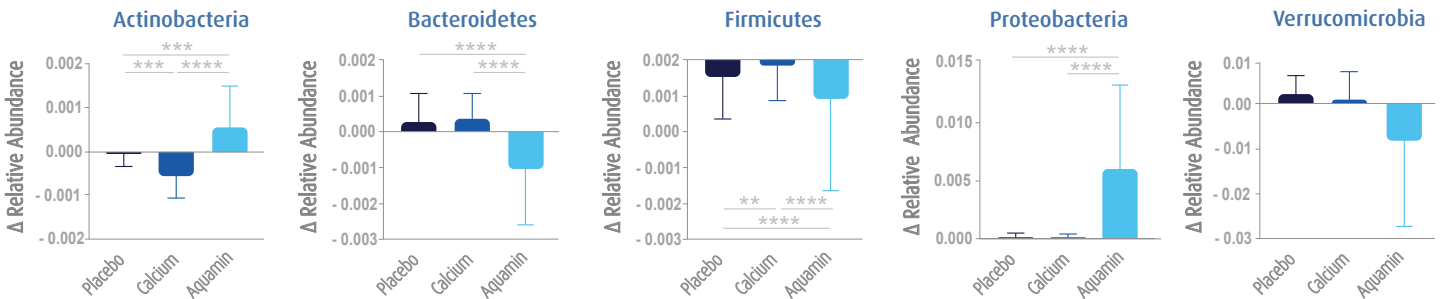


Fig 8: (A) The impact of Aquamin on SCFA profile and (B) the changes in gut microbial communities and diversity (relative abundance at phyla level) after 90 days of consumption.

This study also recorded a reduction in total bile acids and an increase in the level of the SCFAs, (Fig 9). 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 placebo³.

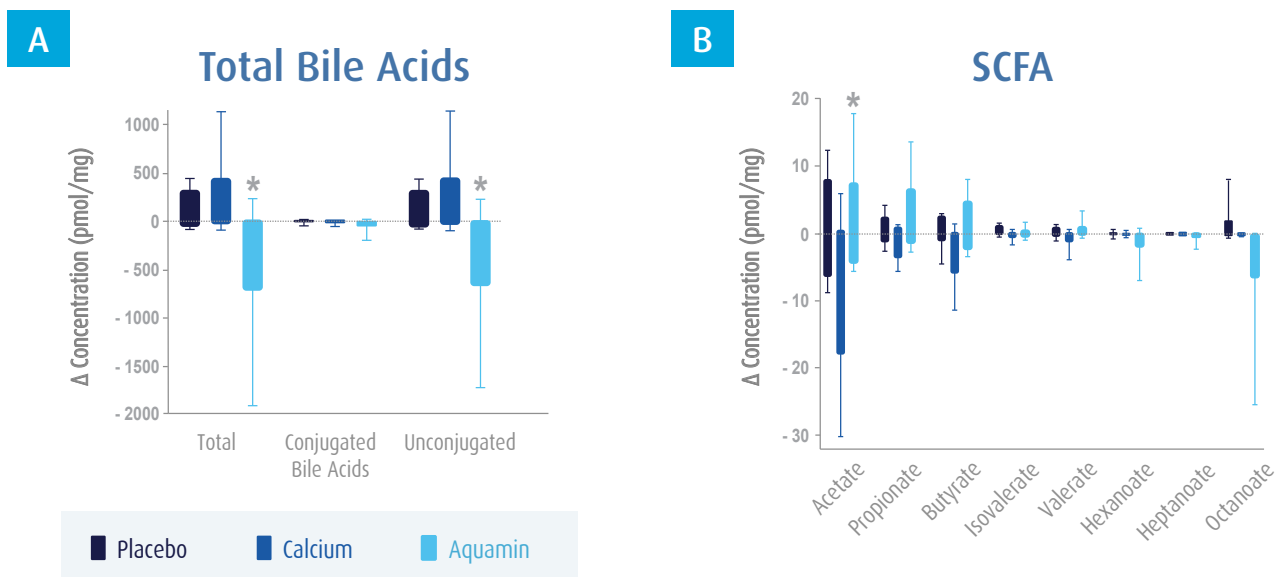


Fig 9: Decrease in bile acids and increase in SCFAs in stool specimens. Values shown represent concentration differences between pre-intervention and post-intervention samples. Asterisks represent statistical significance. (A) Total bile acids (sum of the total conjugated and total unconjugated bile 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 10)¹³. 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.

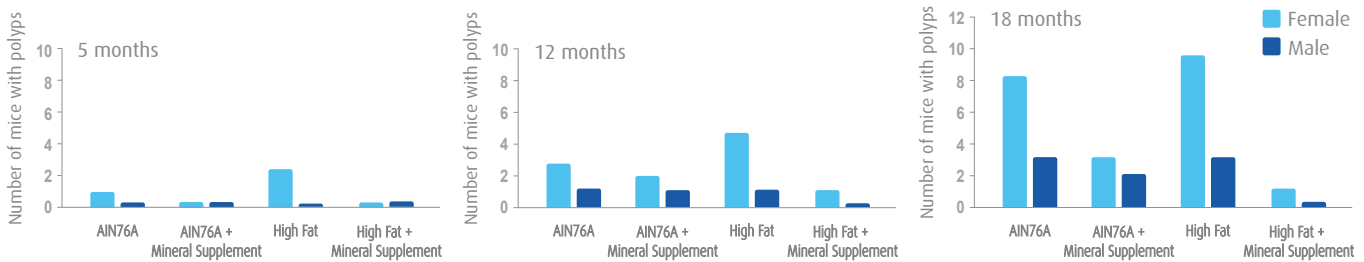


Fig 10: Colon polyp formation in mice fed Aquamin and either a normal or high-fat diet.

Dame et al, 2011¹⁷ 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 phenotype¹⁸. 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 11). However, Aquamin was found to be the most potent in this regard.

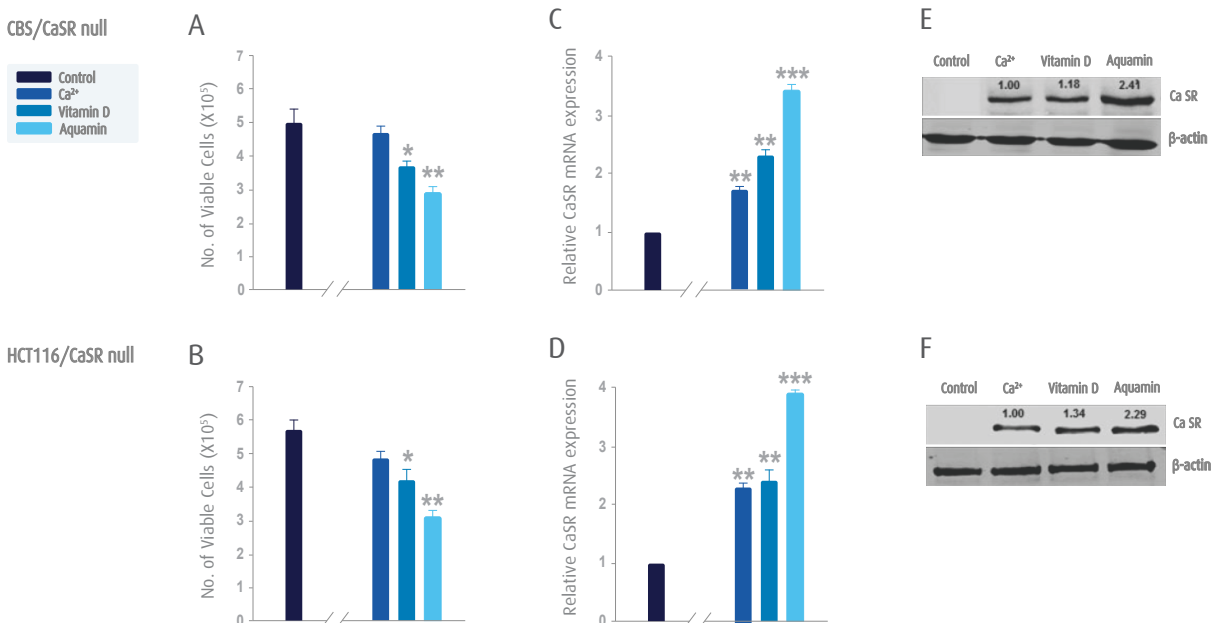


Fig 11: (A) and (B) Inhibition of growth of 2 human colon cancer cell lines by Aquamin. (C) and (D) Induction of Calcium receptor CaSR in 2 colon cancer cell lines which leads to a reduction in cell proliferation. (E) and (F) Western blot of CaSR expression in 2 colon cancer cell lines. Beta-actin used as standard.

Barrier Enhancement

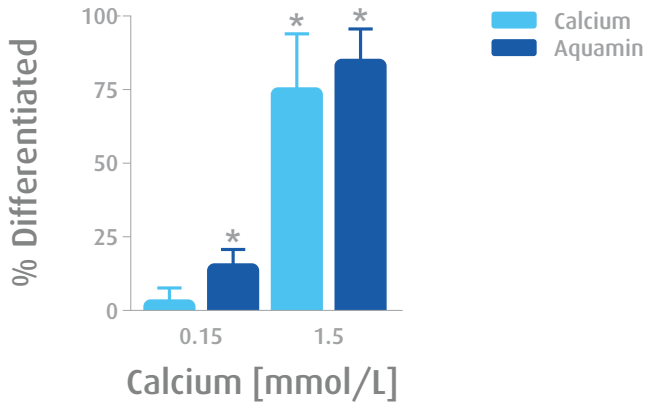


Fig 12: Colonoids maintained in calcium or Aquamin to provide either 0.15 mmol/L or 1.5 mmol/L calcium. Asterisks indicate statistical significance at $P < 0.05$.

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

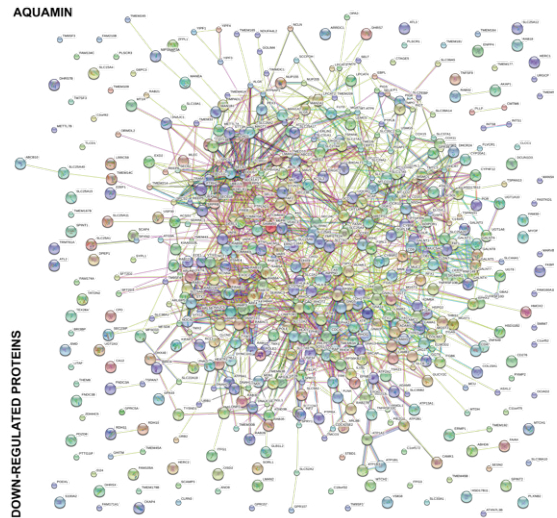
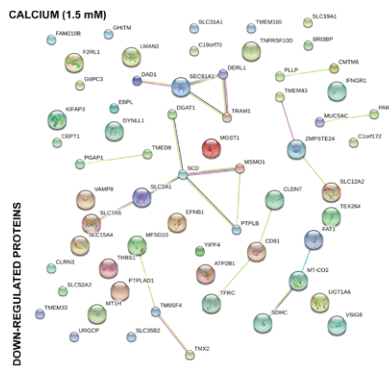


Fig 13: A representation of the proteins either increased or decreased in response to calcium or Aquamin as measured by proteomics.

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.

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 12)¹⁹.

The results showed that Aquamin has a much greater effect on cell proteins than calcium alone (Fig 13) 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., 2019²⁰ where the proteins responsible for membrane impermeability were investigated. As per Fig 14, 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 15: 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.

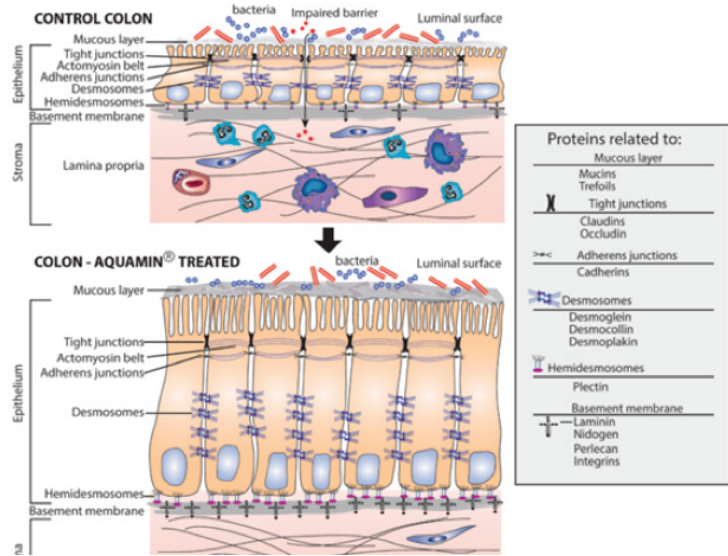


Fig 14: A schematic representation of the role of Aquamin in organising the gut epithelium.

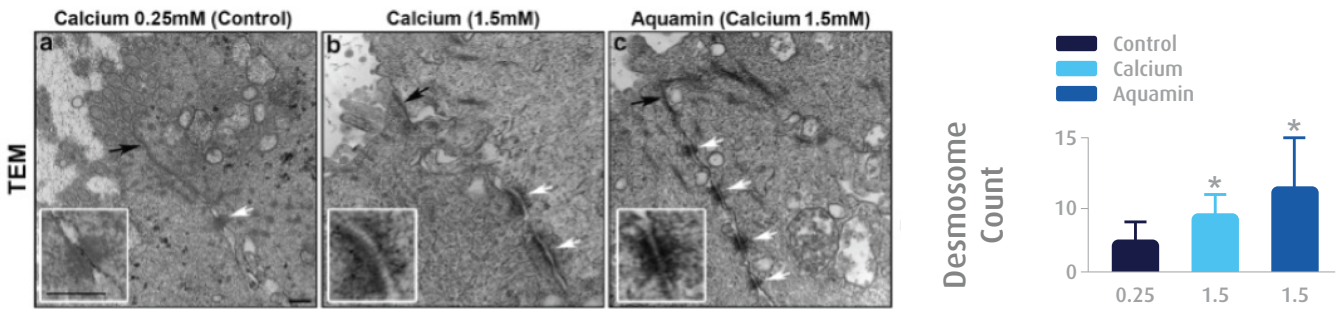


Fig 15: Ultrastructure by transmission electron microscopy. Under all conditions (a-c), tight junctions were evident below the epithelial layer on the luminal side (black arrows). Desmosomes were present in all conditions (white arrows) but a higher density of desmosomes were seen with Aquamin. Asterisks indicate statistical significance from control at $p < 0.05$ level.

Marigot Ltd

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.



Fig 16: Beautiful Bildudalur, the home of Marigot Ltd, Iceland.

For almost 30 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 sustainably and with maximum sensitivity to the environment. From this backdrop, Marigot has created and developed the market for this unique marine multi-mineral, 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 material 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 this unique, natural mineral source. This approach coupled with processing technology, optimised inclusion systems and application development has allowed Aquamin 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. Although numerous anecdotal reports of the health benefits associated with consumption of Aquamin existed, no objective research was carried out until Marigot Ltd collaborated with leading academic institutions to understand exactly how Aquamin could impact human health. Marigot Ltd. have accumulated a large and growing body of research evaluating Aquamin in work spanning in vitro assays, animal models and human trials. Today, there are almost 50 peer reviewed scientific publications supporting the use of this seaweed in human health including digestive health. Marigot is clear about the need for science and the requirement to continuously add value and innovate with this unique ingredient. Extensive research enhances not only the companies knowledge but ultimately that of its customers. Aquamin represents a novel source of natural multi-minerals which has demonstrated efficacy in influencing health. Aquamin will continue to be supported by science and grounded in evidence for those who include it in their diet.

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