



Aquamin

Marine Minerals for Health

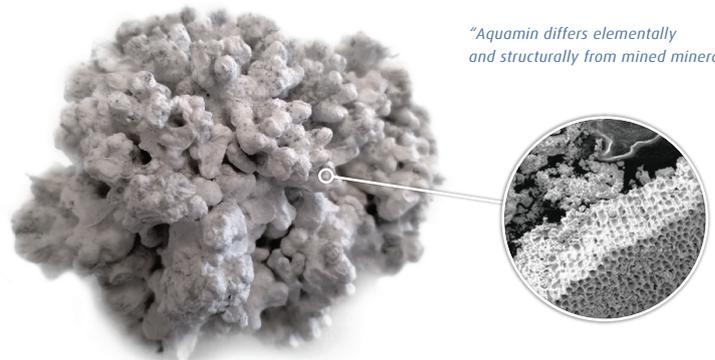
Aquamin & Gut Health



Summary

Aquamin™ is a unique natural ingredient for the enhancement of gut health. It contains significant amounts of calcium and magnesium as well as trace amounts of 72 additional minerals complexed together in a structure engineered by the cell wall of the seaweed *lithothamnion* sp. Aquamin has been the subject of 33

peer reviewed publications over the last 10 years, which support its unique health promoting properties. Aquamin has been shown to enhance gut health by restoring a balanced immune response, promoting the differentiation of colonic cells and providing a balanced gut microflora



Overview

Aquamin is derived from the cytoskeleton of the red seaweed *Lithothamnion* sp. Over the course of the seaweed's life, minerals are accumulated from the surrounding seawater, and are stored as carbonate salts in the plant's cell wall. Calcium and magnesium represent one third and over 2%, respectively of the 74 components in the total dry mass of Aquamin. It displays an intricate and unique 3-dimensional structure moulded by the cell wall of the plant. This unique feature is associated with the novel bioactivities attributed to Aquamin when compared with other sources of multi-mineral complexes.

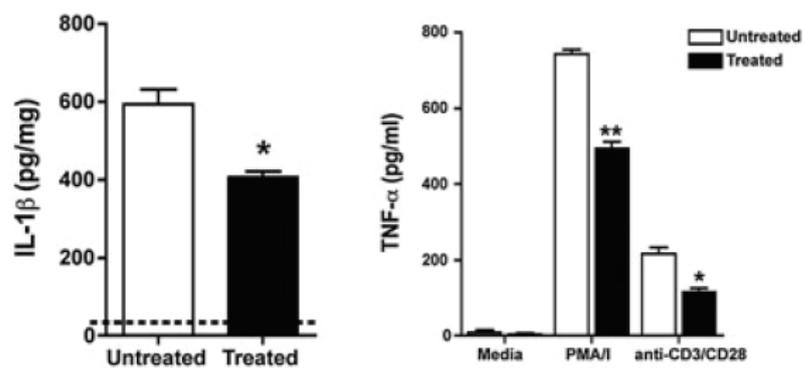
The development of consumer demand for Aquamin was pioneered by Marigot Ltd, by creating awareness and providing innovative research data supporting the inclusion of the 74 marine minerals beneficial for human nutrition. During this time, extensive human studies ensued in an effort to fully evaluate 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 an exclusive 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 arctic, thus 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 sustainable manner and that this precious resource is protected for future generations. An extensive quality control programme ensures traceability, quality and consistency for each batch of Aquamin.

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. This 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.



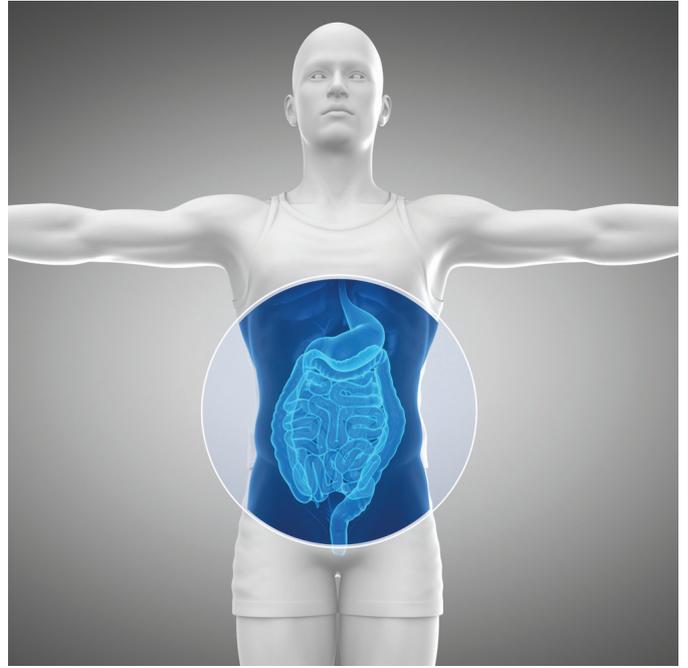
The Impact of Aquamin (Treated) on 2 markers of active inflammation in Colitis. PMA/I and antiCD3/CD28 represent different subsets of immune cells from the spleen.

In the area of gut health Aquamin has been demonstrated to provide:

- ✓ Improvement of the symptoms of colitis
- ✓ Anti-inflammatory activity demonstrated in the GI tract
- ✓ Protection from liver damage while on a high fat Western diet
- ✓ Prevention of polyp formation on a high fat Western diet
- ✓ Maintenance of healthier stomach wall on a high fat Western diet

Aquamin and 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, testing the hypothesis that the absence/reduced intake of multi-minerals in a Western style diet, may contribute to diseases with a diet associated factor for exacerbation/incidence. These investigations began in-vitro, with preliminary observations showing that cultured gut-lining (epithelial) cells showed improved differentiation (non-malignant effect) and proliferation (healthier cells) in the presence of Aquamin (Aslam et al., 2009)¹ 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)² 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)³ An incidental finding from the study was a significantly reduced liver mass formation in mice on the Western Style Diet and fed Aquamin versus the controls and limestone derived calcium (Aslam et al., 2012)⁴ Taken together, these results have prompted Marigot Ltd, alongside colleagues from the University of Michigan, to investigate whether these anti-inflammatory effects in-vitro and in the digestive tract of mice can also be observed in humans. This FDA approved and regulated trial is currently underway.

The beneficial effects of Aquamin were also seen in a mouse model of colitis. Colitis is one of a number of 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 (Aviello et. al., 2014)⁵

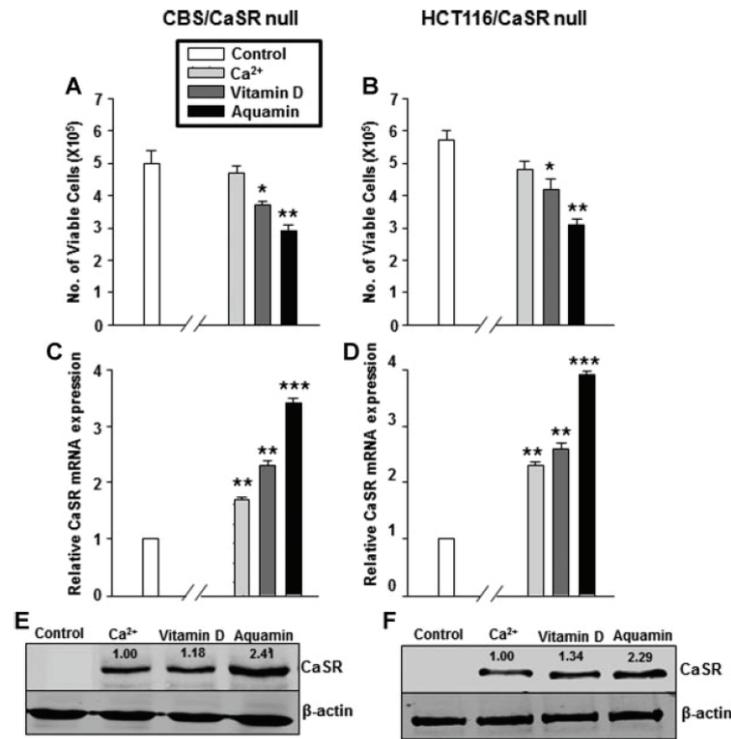
Aquamin and Gut Microbial Population

A growing amount of scientific evidence suggests that colon microbiota of Irritable Bowel Syndrome (IBS) patients differ from that of healthy subjects (Kassinen et al., 2007)⁶ 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 and 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 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 will be further investigated in dietary intervention studies.

Aquamin Polyp Formation and Gut Malignancy

The level of colon polyp prevention seen with Aquamin is clearly greater than previously evidenced for calcium alone, which leads to the strong argument that these 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 formations or suppress other types of tissue injury in gastro-intestinal tracts. It is also 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 Aquamin, are able to reduce colon polyp formation in high-fat and low-fat diets. 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.



(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.

(Dame et al, 2011)⁷ showed that treatment of human colon tissue in organ culture with Aquamin is sufficient to see immunohistochemical changes reflective of improved differentiation and appears to enhance 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)⁸. 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. However, Aquamin was found to be the most potent in this regard. This study provides a rationale for the use of a multi-mineral approach for a healthy gut.

Marigot Ltd

Marigot Ltd. was established in 1993 by Les Auchincloss, previously founder and major shareholder of Biocon Limited until 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 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.

Marigot is clear about the need for science – the requirement to continuously add value and innovate with this unique ingredient through extensive research thereby enhancing 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.



In May 2016, Marigot Ltd was awarded Ingredient of the Year (Healthy Ageing) at the Nutraingredients awards at Vitafoods, Geneva. This was in recognition of the strong body of scientific trial data that supports Aquamin and the positive contribution it has made to health.

References

1. Aslam MN, Bhagavathula N, Paruchuri T, Hu X, Chakrabarty S, Varani J. Cancer Letters.(2009) Oct 8;283(2):186-92. Growth-inhibitory effects of Aquamin, a mineralized extract from the red algae, Lithothamnion calcareum, on Ca²⁺-sensitive and Ca²⁺-resistant human colon carcinoma cells.
2. Aslam MN, Paruchuri T, Bhagavathula N, Varani J., Integr Cancer Ther (2010) 9:93. A Mineral-Rich Red Algae Extract Inhibits Polyp Formation and Inflammation in the Gastrointestinal Tract of Mice on a High-Fat Diet.
3. Aslam MN,, Bergin I, Naik M, Paruchuri T, Hampton A, Rehman M, Dame MK, Rush H, Varani J. Nutrition and Cancer,(2012) 64 (7): 1020-8. A Multimineral Natural Product from Red Marine Algae Reduces Colon Polyp Formation in C57BL/6 Mice.
4. Aslam MN, Bergin I, Naik M, et al. A multi-mineral natural product inhibits liver tumor formation in C57BL/6 mice. Biol Trace Elem Res. 2012;147(1-3):267-74.
5. Aviello G, Amu S, Saunders SP, Fallon PG. A mineral extract from red algae ameliorates chronic spontaneous colitis in IL-10 deficient mice in a mouse strain dependent manner. Phytotherapy research : PTR. 2014;28(2):300-4.
6. Kassinen, A., et al., The fecal microbiota of irritable bowel syndrome patients differs significantly from that of healthy subjects. Gastroenterology, 2007. 133(1): p. 24-33.
7. Dame MK, Veerapaneni I, Bhagavathula N, et al. Human colon tissue in organ culture: calcium and multi-mineral-induced mucosal differentiation. In Vitro Cell Dev Biol Anim. 2011;47(1):32-8.
8. Singh N, Aslam MN, Varani J, Chakrabarty S. 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. Mol Carcinog. 2015;54(7):543-53.