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Rejuvenating effect of snow algae analysed

ABSTRACT

Snow algae powder is a novel anti-ageing ingredient based on an extract of biotechnologically produced snow algae. In cell culture assays, the snow algae extract was found to induce a calorie restriction-mimetic effect by stimulating the expression of the Klotho gene and the activity of the AMPK protein. The extract reduced the loss in collagen expression in aged fibroblasts and counteracted the increase in matrix metalloproteinases in senescent fibroblasts. In clinical studies, the snow algae powder was shown to improve the papillary structure of the dermal epidermal junction, significantly enhanced skin hydration and smoothed crow's feet wrinkles.

In summer, persisting snow fields on high mountains are sometimes tinged with a red, pink colour (Fig. 1). This phenomenon is caused by snow algae. These are unicellular members of green algae that changed colour by producing carotenoids for protection against the high ultraviolet (UV) radiation. Snow algae are a species adapted to survive the extreme conditions of alpine and polar snow fields. The resting algae containing high amounts of astaxanthin carotenoids are deposited when the snow melts and stay there during the winter under the newly formed snow layer. In spring when the snow partially melts at around 0˚C, the red algae change into green organisms that move with the aid of flagella to the surface of the snow layer. There sunlight causes photosynthetic metabolism resulting in a short reproduction period. In summer, when the UV radiation is too high, the algae revert to the immobile, carotenoid-containing resting forms.

A tube photobioreactor (air-lift mode) was developed to produce snow algae raw material from a *Chlamydocapsa* species (Fig. 2). In a first stage, optimal light and aeration was used to grow the green algae for three weeks doing photosynthesis in order to reach biomass. In a second stage, nutrients were reduced and light strongly increased over two weeks to induce the formation of carotenoids and thus the red coloured resting form. Snow algae harvested at this stage were homogenised at 1200 bar in a phospholipid solution to disrupt the cells and to form liposomes with encapsulated water- and oil-soluble algae actives (snow algae extract; INCI: Chlamydocapsa sp. – 101 Extract). The liposomal extract was carefully coated onto maltodextrin in a spray granulation process (snow algae powder; INCI: Chlamydocapsa sp. – 101 Extract, Maltodextrin, Lecithin, Water).

Preparations of the snow algae were tested for anti-ageing effects in cultures of skin cells (snow algae extract) and in clinical studies (snow algae powder). Surprisingly, the algae extracts were found to interfere with the insulin/IGF-1 and the AMP-activated protein kinase (AMPK) signalling, two pathways involved in the calorie restriction-induced longevity phenomenon. The insulin/IGF-1 signalling pathway is a cellular sensor for nutrients (Fig. 3). Under high nutrient and insulin conditions, the receptor gets phosphorylated, leading to inactivation of the Forkhead transcription factor FOXO inside the cell. Under low-nutrient conditions, this signalling pathway is blocked and as a consequence, the FOXO gets activated causing the cellular metabolism to focus on protection, repair and efficiency leading finally to longevity.

Figure 1: *Red spores of snow algae in a high mountain snow field.*

Suppression of the insulin/IGF-1 signalling pathway is regarded as the central mechanism in the calorie restriction-induced longevity phenomenon.[1] The AMPK is a cellular sensor for energy which is activated by an increased AMP/ATP ratio indicating low energy. During calorie restriction and after exercise AMPK activity is increased to restore the ATP level by stimulating ATP-generating processes and by inhibiting ATP-consuming processes that are not needed for survival. But the role of AMPK is not restricted to the control of the energy metabolism. AMPK is a type of master switch that was shown to regulate several transcription factors related to longevity and ageing.[2] AMPK can activate FOXO and Nrf2, another activator of the skin's own defence systems. AMPK blocks NF-κB and thus inhibits inflammatory reactions. Stimulation of AMPK activity induces anti-ageing effects and confers longevity.

Methods

In the replicative ageing study, aged (passage 17) primary human fibroblasts were incubated for 24 hours in a culture medium containing or not (control) 1% of the algae extract. After incubation, the expression of 64 genes related to stress and ageing was analysed by quantitative PCR technology (LightCycler system Roche Molecular System Inc.). Young (passage 8) primary human fibroblasts were also incubated in normal culture medium and analysed for gene expression (control 2).

In the premature senescence study, primary human fibroblasts were stressed

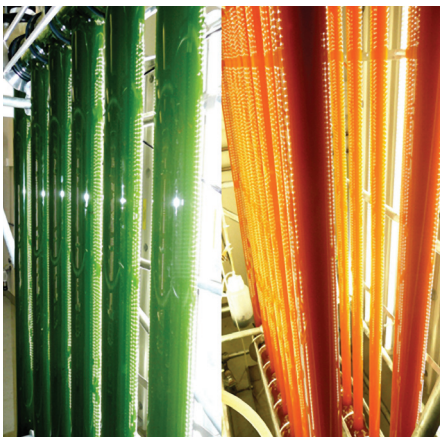

Figure 2: *Tube photobioreactor with snow algae in the green propagation phase (high nutrients, normal light) and in the red duration phase (low nutrients, strong light).*

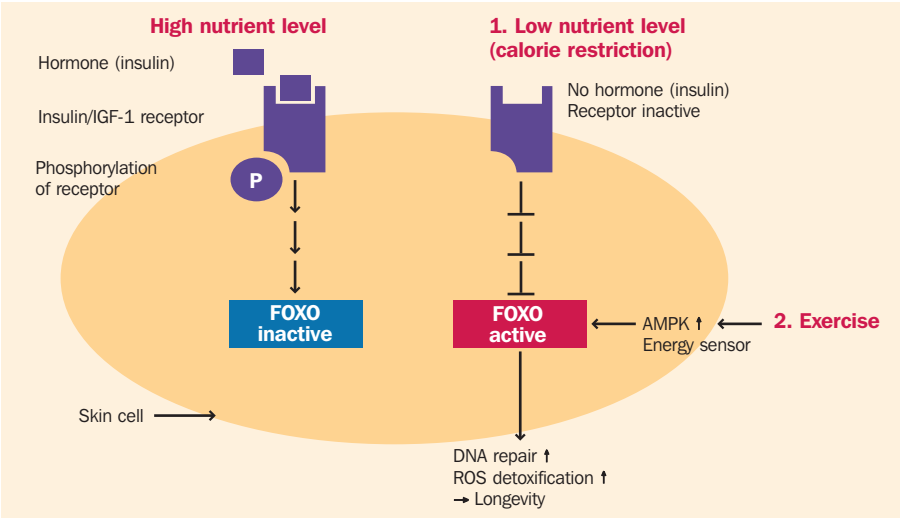

Figure 3: *The insulin/IGF-1 receptor as nutrient sensor and AMPK as energy sensor.*

for 2 h in a culture medium containing 600 µM H_2O_2. After a recovery incubation period, the fibroblasts were incubated for 24 hours with culture medium containing 1% of the algae extract and also in a control medium. After incubation, the expression of 64 genes related to stress was analysed by quantitative PCR technology (LightCycler system Roche Molecular System Inc.).

The effect of the algae extract on AMPK phosphorylation was tested in a study with primary human keratinocytes in an assay medium supplemented with insulin (60 ng/mL). The keratinocytes were incubated for 48 hours in an assay medium containing or not (control) 1% of the algae extract. The amounts of total AMPK and phosphorylated AMPK were determined by Western blot analysis.

The clinical study to analyse the papillary structure of the dermal epidermal junction was performed with five women aged 55-67. A cream with 2% snow algae powder was applied twice daily for eight weeks to the inner side of the forearm. The placebo cream was applied on the other forearm. To perform multi-photon microscopy, image stacks were acquired using the Olympus Fluoview 1000 MP two-photon microscope. Images were obtained simultaneously in two modes, autofluorescence (AF) and second harmonic generation (SHG), with the following acquisition setup – illumination at 800 nm, femtosecond laser emission filters:

- Channel 1 – band pass 397-412 nm (SHG, colour-coded red)
- Channel 2 – band pass 455-490 nm (AF of elastic fibres, NAD(P)H, colour-coded green)

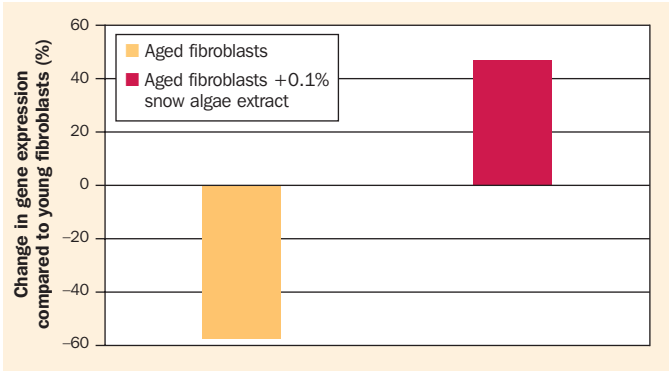

Figure 4: *Stimulation of klotho gene expression in aged fibroblasts.*

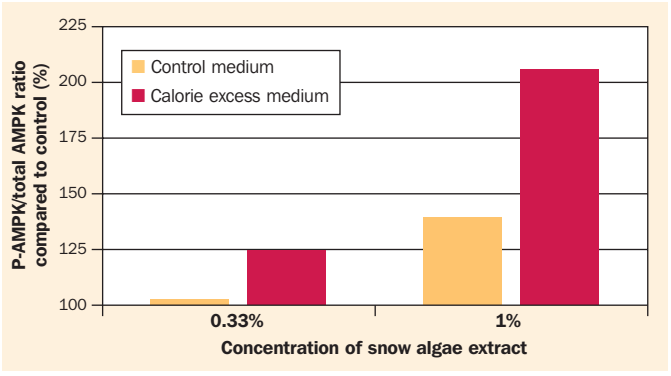

Figure 5: *Stimulation of phosphorylation (activation) of AMPK in normal medium and under calorie excess conditions.*

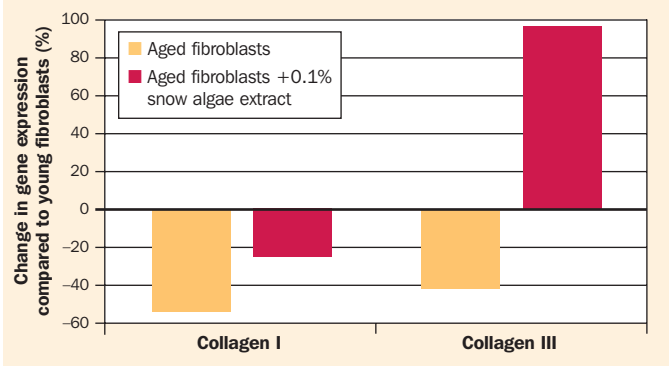

Figure 6: *Activation of collagen I and III expression in aged fibroblasts.*

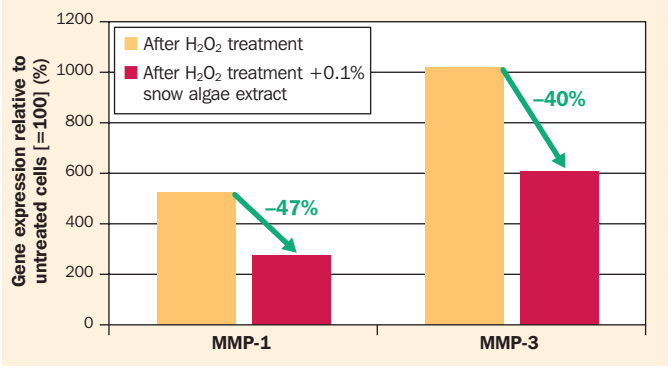

Figure 7: *Inhibition of the up regulation of MMP-1 and -3 expression in fibroblasts after H_2O_2 treatment.*

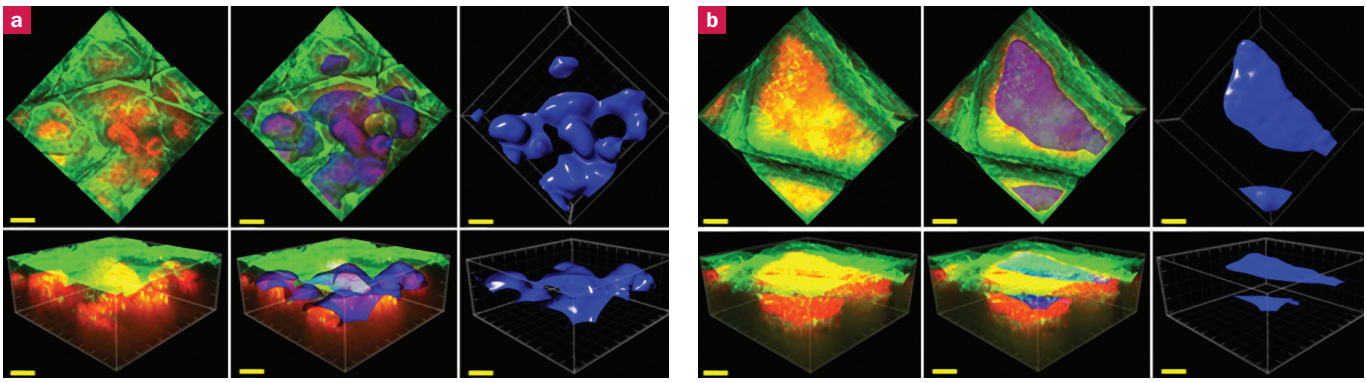

Figure 8: *Representative three-dimensional two-photon microscopy images and calculated papillary surface pictures of* **a)** *young female skin (31 years) with convex papillae and normal basal membrane area; and* **b)** *aged female skin (67 years) with smoothed, flattened papillae and decreased basal membrane area.*

- Channel 3 – band pass 526-577 nm (AF of FAD)

The clinical study to measure skin hydration and wrinkles was performed with 20 women aged 40-60. A cream with 2% snow algae powder was applied twice daily for 28 days to the inner side of the forearm and to the crow's feet area. The placebo cream was applied to the other side. At days 0, 14 and 28, hydration was measured with the Corneometer MPA 5 CPU (Courage & Khazaka GmbH, Germany) and wrinkle depth with the PRIMOS 5.7 high-res (GFMesstechnik GmbH, Germany).

Results and discussion

Stimulation of the longevity gene Klotho

In a replicative ageing model with primary human fibroblast cells, the expression of the Klotho gene was found to be down-regulated in aged cells (Fig. 4). Treatment of aged cells with the snow algae extract induced an up regulation of Klotho expression to a value even beyond that of young cells. Klotho, named after a Greek goddess who spins the thread of human life, is a longevity-related gene, discovered in 1997. Mice deficient in Klotho display an accelerated ageing phenotype and on the other side when overexpressed, the gene extends lifespan by 30%.[3] There are two forms of the Klotho protein known: transmembrane Klotho acting as a co-receptor of fibroblast growth factor 23 and secreted Klotho acting as a hormone. The latter was found to act by inhibiting the insulin/IGF-1 signalling at the level of the insulin receptor substrate protein.[4] Independent of the nutrient and insulin levels Klotho inhibits phosphorylation of the insulin/IGF-1 receptor. Stimulation of Klotho to block the insulin/IGF-1 signalling pathway thus induces a calorie restriction-like cellular metabolism.

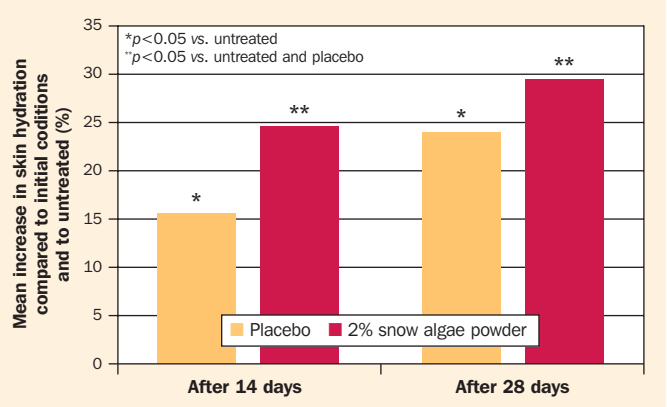

Figure 9: *Placebo-controlled, statistically significant improvement of skin hydration.*

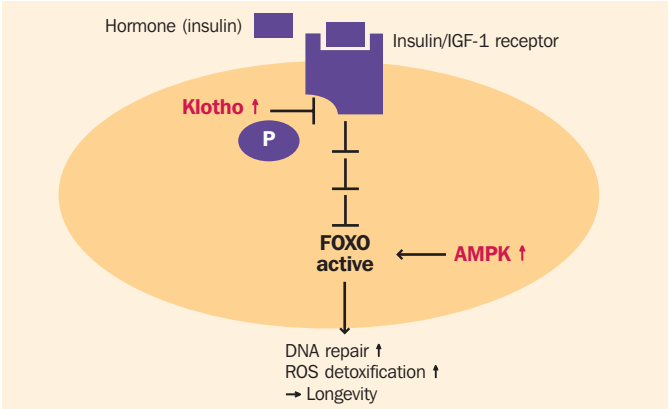

Figure 11: *Inhibition of insulin/IGF-1 receptor phosphorylation by the klotho protein and stimulation of AMPK activity to induce calorie restriction-mimetic longevity.*

Activation of the master switch AMPK

The effect of the snow algae extract on phosphorylation (activation) of AMPK was tested on human primary keratinocytes under normal conditions and in the presence of high insulin concentrations representing high nutrient conditions. Expression of AMPK-regulated genes is repressed under high nutrient conditions leading to a pro-ageing situation. The snow algae extract was found to stimulate phosphorylation of AMPK, especially under high nutrient conditions, by 105% in this case (Fig. 5). Stimulation of AMPK activity by the snow algae extract mimics a situation of low calories and energy similar to that after doing exercise.

Stimulation of collagen production and inhibition of its breakdown

In the replicative ageing model with primary human fibroblast cells, the expression of the collagen genes I and III was found to be down-regulated in aged cells (Fig. 6). Treatment of aged cells with the snow algae extract induced an up-regulation of collagen expression leading to a correction for collagen I and even to a higher expression of collagen III compared to young cells. Using a premature senescence model with primary human fibroblast cells, the expression of the collagen-degrading enzymes matrix metalloproteinase 1 and 3 was found to be greatly up-regulated after H_2O_2 exposure (Fig. 7). After incubation with the snow algae extract, the up-regulation could be reduced by 47% and 40% respectively.

Improvement of the papillary structure

In another clinical study, two-photon microscopy was used as a novel non-invasive method to analyse papillary surface area and collagen to elastin ratio. Two-photon microscopy makes skin imaging possible deep in the skin (upper dermis). Near infrared wavelengths are used to build up a tissue contrast based either on auto-fluorescence generated for example by elastin and NADH or based on second harmonic generation induced by collagen structures. The papillary surface, corresponding to the surface of the basal membrane, can be reconstructed using special software and algorithm. Figure 8 shows example pictures from a young and old female volunteer. A cream with the extract was applied by five women aged 55-67 to the inner side of the forearm for two months. The placebo cream was applied to the other forearm. At the end of the study, the papillary surface was increased by 12.5% compared to initial conditions and by 30.5% compared to the placebo. A rejuvenation effect was also shown by the placebo-controlled increase in the collagen to elastin ratio (+12%).

Improvement of skin hydration and smoothing of eye wrinkles

A basic o/w emulsion with 2% snow algae extract was found to strongly improve skin hydration, already after two weeks application (Fig. 9). The effect was significantly better than that of the placebo cream. Wrinkle depth in the crow's feet area was improved by 10% on average but up to 29%. Before and after 3D visualisation pictures of a volunteer are shown in Figure 10.

Conclusion

Snow Algae Powder is a new cosmetic ingredient based on a novel anti-aging mechanism. By stimulating expression of the Klotho gene and AMPK activity, it induces a calorie restriction-mimetic effect (Fig. 11). This will lead to longevity of skin cells because repair and detoxification systems are up regulated and the cellular energy metabolism works at its highest efficiency. The snow algae powder interferes with intracellular pathways and only mimics calorie restriction as the skin is well supplied with nutrients and energy all the time. Results of clinical studies demonstrated real skin benefits for the calorie restriction-mimetic activity of the snow algae powder: the papillary structure of the dermal epidermal junction was rejuvenated, skin hydration improved and eye wrinkles smoothed. PC

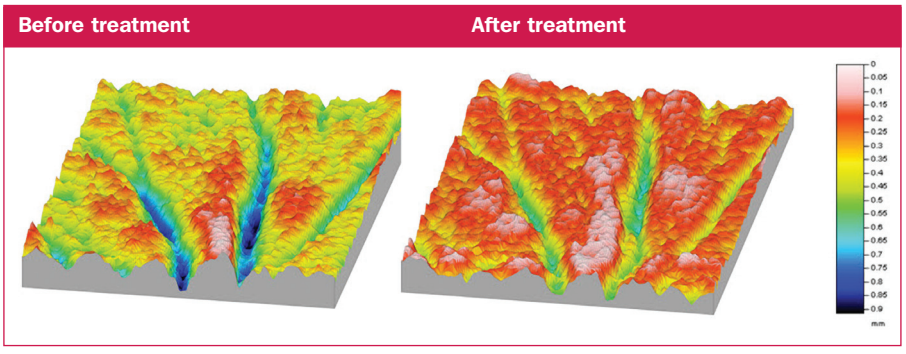

Figure 10: *Representative before/after three-dimensional visualisation of crow's feet wrinkles.*

References

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