mibelle GROUP biochemistry

Snow Algae Powder

Key to skin’s longevity

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The Secret of the Red Snow

- Sometimes a red colour in the snow can be discovered
- Mainly in summer, sun exposed snow slopes can show a redish colour
- Colour comes from spores of snow algae
- The red colour protects the algae against extreme UV-radiation
- Today about 350 species of snow algae are known.

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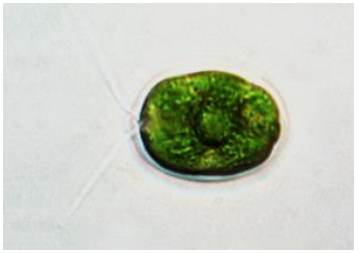
Snow Algae -
Life Cycle with two Phases



In spring
Snow algae are buried under the snow and appear green (chlorophyll pigments to produce energy)
→ movement to snow surface (flagella)
→ snow algae propagate

Early summer
Snow algae reach the surface
→ strong UV light, low nutrients
→ algae turn red (spores)

In the form of spores, the snow algae survive under a blanket of snow until next spring.

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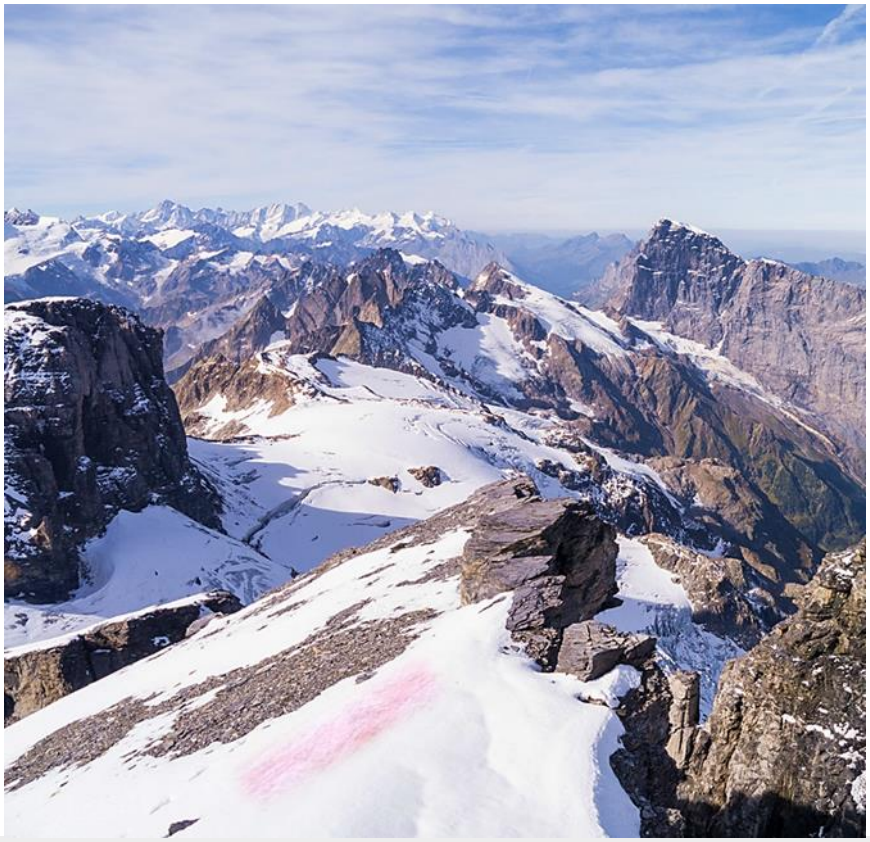
Snow Algae Live under Extreme Conditions



- Extreme temperatures, strong UV-radiation, lack of nutrients
- Snow algae live on water, CO_2, sun light and minerals
- Growth rate is highest at temperatures between 0 and +5° C

→ Extreme habitat: snow algae have developed a series of adaptations that are reflected by diverse secondary metabolites

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Green- and Red Stage of Snow Algae

Growth and reproduction stage

- Chlorophyll (photosynthesis → energy)
- Flagella

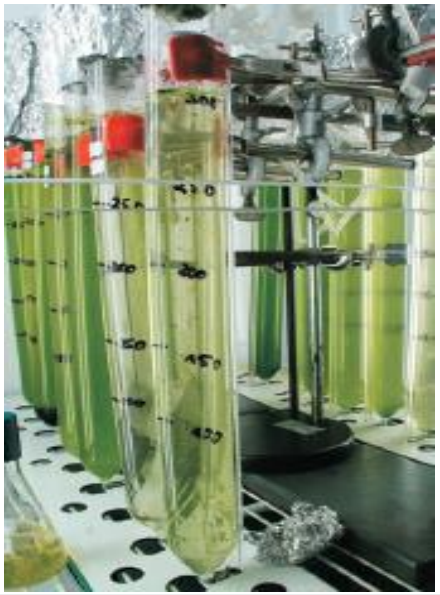
↓ Stress:
UV, lack of nutrients

Dormant stage (spores)

- Carotinoids
- Antioxidants
- Polyphenols
- Biopolymers (gallerten)
- Antifreez glycoproteins (AFGPs)
- Osmotically active aminoacids and sugars

→ Interesting metabolites

Harvesting of Snow Algae, Breeding in the Lab and Biomass Production



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Production of Snow Algae Powder

Favourable conditions

- High nutrition
- Light

Algae are green (chlorophyll) and propagate

Changing the conditions

- Nutrients are reduced
- Light is strongly increased

Algae produce carotenoids to protect against light, cells stop growing

Harvesting

Disruption of the cell walls, encapsulation of cell fragments and content into liposomes.

Spraying on a powder based on maltodextrin.

Snow Algae Powder Composition

Snow Algae Extract (dry)	0.2%
Maltodextrin	92%
Phospholipids	0.16%
Aqua (residual moisture)	~7%

INCI* (EU-Declaration / PCPC-Declaration)

Chlamydocapsa sp.-101 Extract * (and) Maltodextrin (and) Lecithin (and) Aqua/Water

*INCI not yet confirmed

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Snow Algae Powder
A New Cosmetic Ingredient

- Unique source
- Produced by biotechnology (sustainability)
- With a novel activity:

→ Caloric restriction mimetic anti-aging

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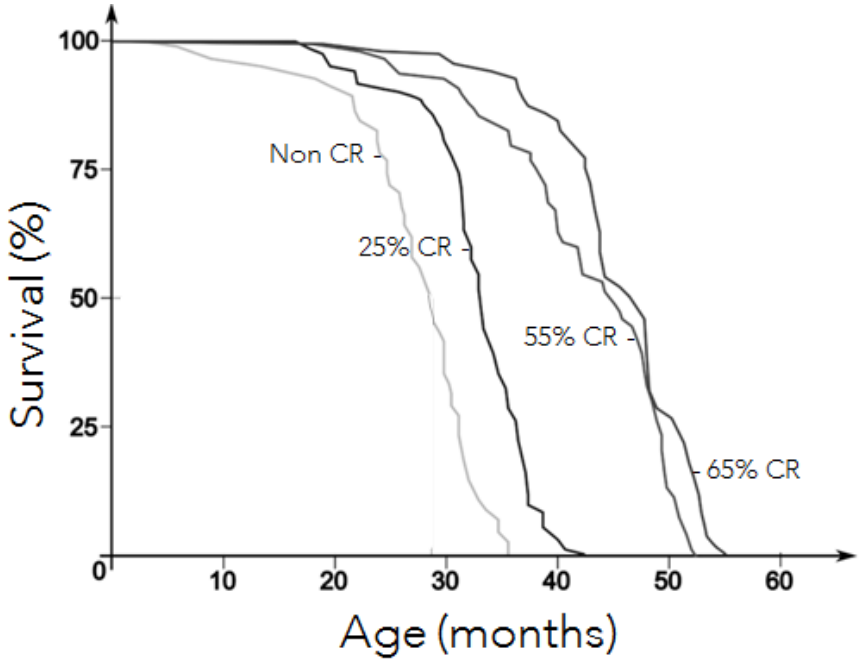
Caloric Restriction (CR) Increases Lifespan in Rats and Mice



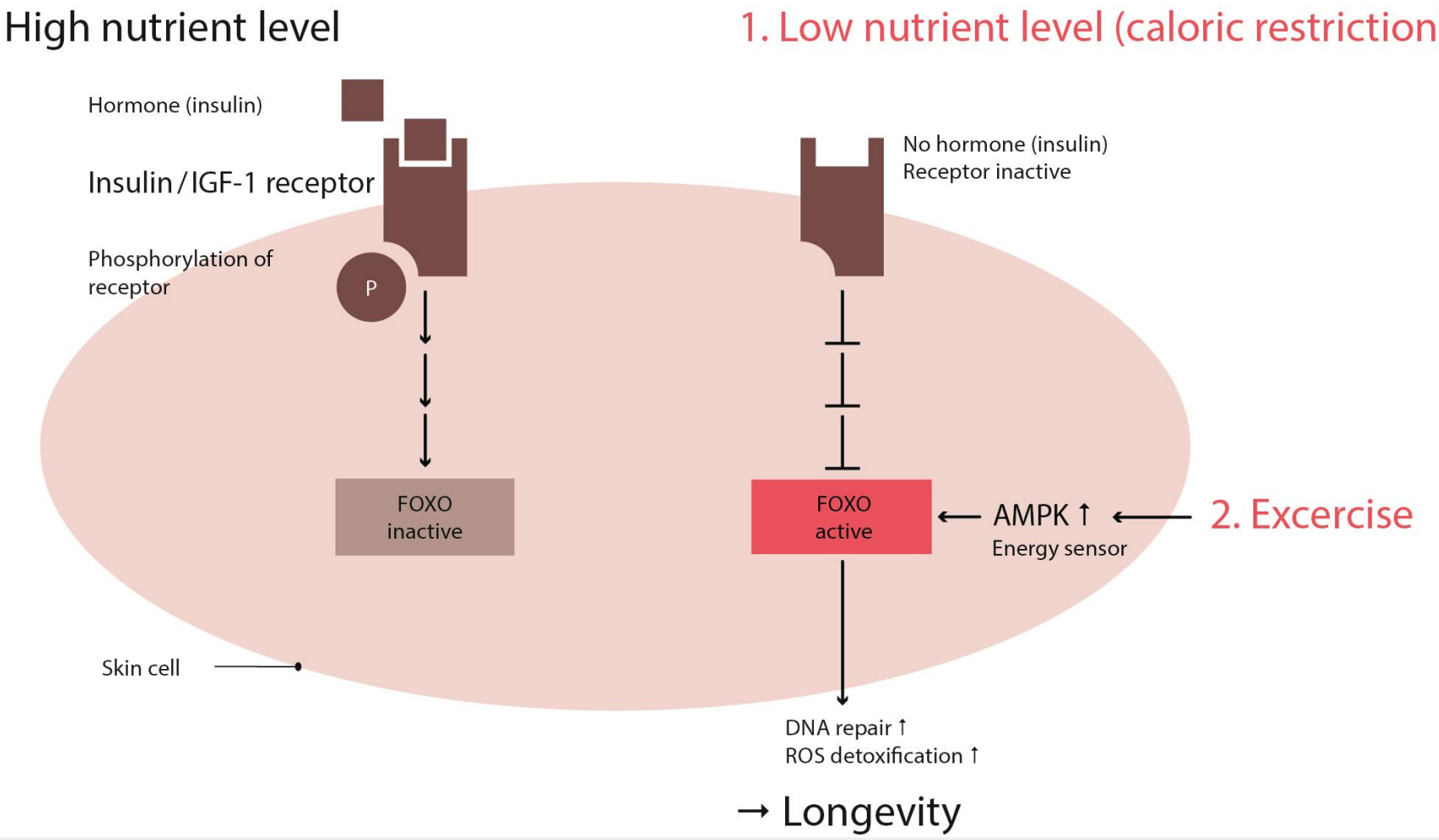
- 1934 Mary Crowell and Clive McCay observed a significant increase in lifespan under caloric restriction with rats.
- Similar results in mice (Weindruch R, et al.,1986).

Data taken from: Weindruch R, et al. (1986). Journal of Nutrition, April, 116(4), 641-54.

Secret of a Long Life

- Life extension in yeast, worms, mice, rats, dogs, apes and men?
- Food limitation (world war) → increase in lifespan
- Fasting cures (religions) → health benefits
- 5:2 diet → weight loss and health benefits
- Okinawa residents with low energy diet → large number of centenarians

The Caloric Restriction and Longevity Pathway



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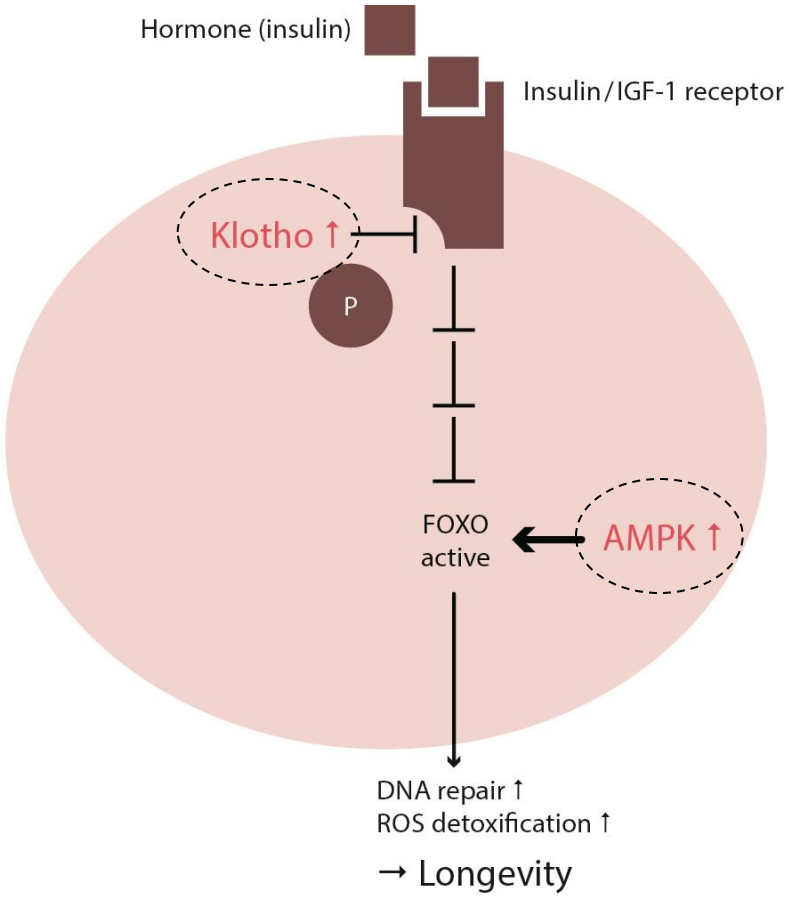
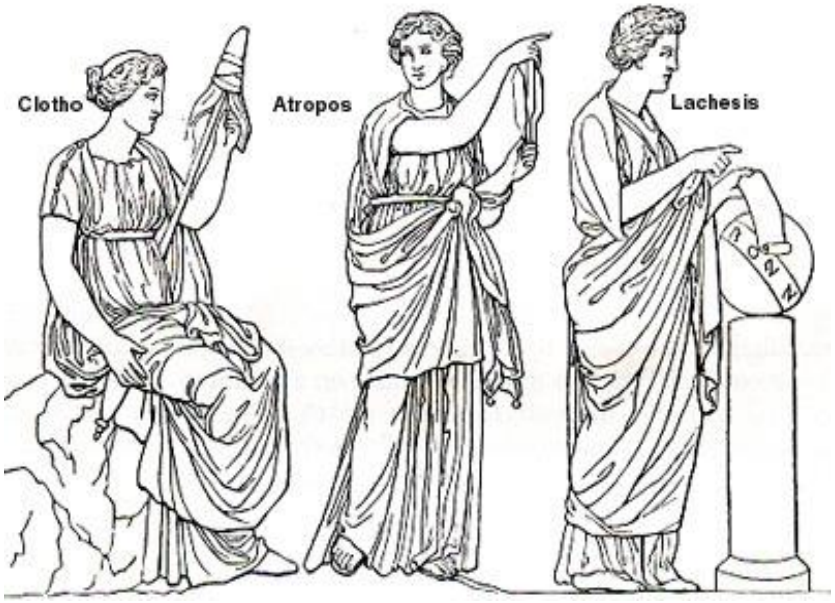
Two Key Factors Stimulate the Longevity Pathway



1. Klotho ↑
Insulin/IGF1-receptor is blocked
FOXO stays active
→ Longevity

2. AMPK ↑
FOXO further activated
→ Longevity

1. Klotho
A Button for Longevity



- In 1997 a gene was identified in mice that caused the **extreme aging** signs when it is damaged.
- The gene was named **Klotho**, for the Greek goddess of fate.
- When overexpressed **Klotho** extends lifespan.
- **Klotho** represses intracellular signals of insulin and insulin-like growth factor 1 (IGF1).

2. AMPK
AMP- Activated Protein Kinase

- Master regulator of metabolism
- AMPK is activated
  - when energy (ATP) is at a low level (e.g. caused by CR)
  - upon exercise

- AMPK activity declines
  - when we age
  - upon nutritional overload

  → increased oxidative stress

Longevity and Mutations

Insulin / IGF-1 receptor mutants

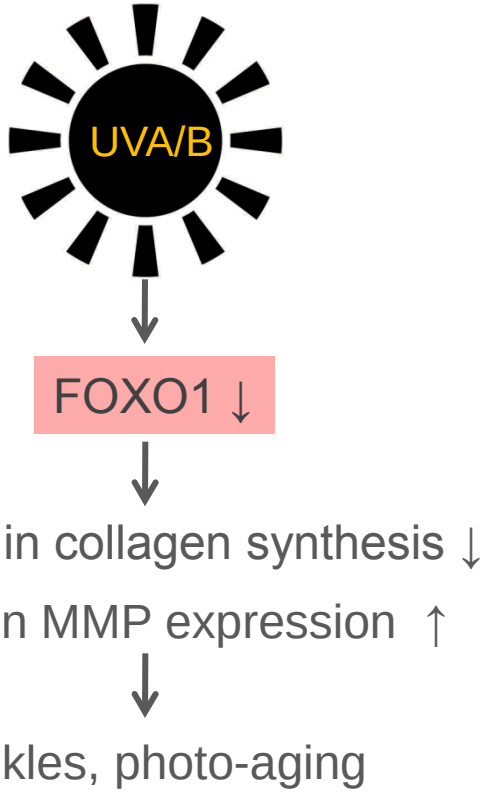
- horses and dogs → smaller dogs and horses live longer
- Japanese cohorts with longevity

FOXO mutations

- 8 independent cohorts around the world of long-lived subjects

UV Radiation and FOXO in Skin Aging

UVA /UVB irradiated fibroblasts show a decreased expression of FOXO1:



Tanaka, H. et al., Journal of Investigative Dermatology Symposium Proceedings (2009): 14, 60-62.

Mimicing Caloric Restriction: New Anti-Aging Concept

Caloric restriction (CR) and exercise can extend life-span and health-span:

→ Search for actives which **mimic** the biochemical effects of caloric restriction

→ Extension of health-span and life-span of skin cells without diet and exercise

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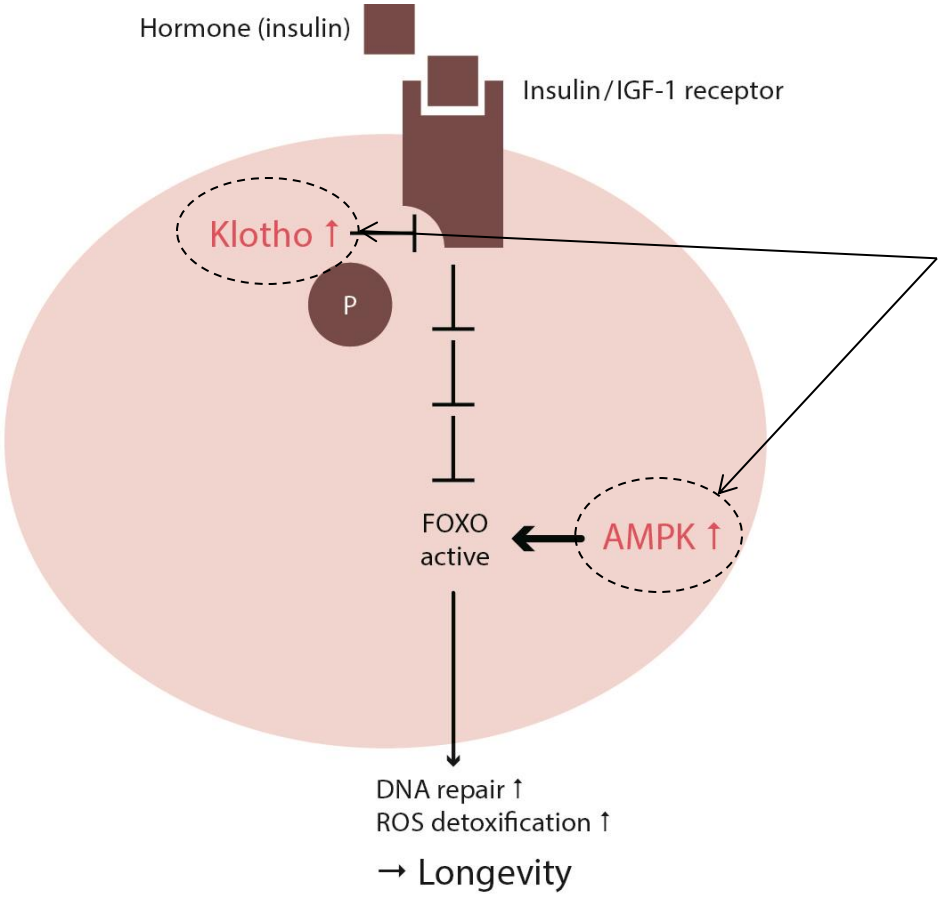
Screening for CR Mimetic Anti-Aging: High Klotho and AMPK Activity



Goal

Find an active ingredient that increases

1. Klotho anti-aging activity
2. AMPK activity

in aged skin

= CR mimetic activity

1. Klotho Anti-Aging Activity

Cell type

Normal human dermal fibroblasts (NHDF)

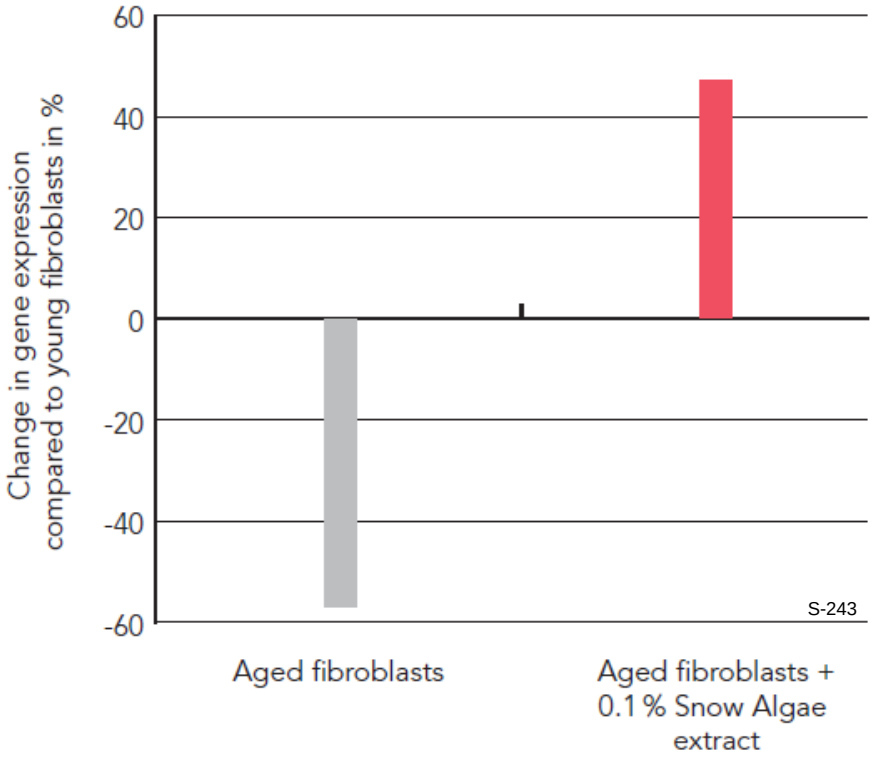
Study design

Comparison of NHDF at passage 8 (young) and at passage 17 (aged fibroblasts)

Parameter

Activity of Klotho by quantitative PCR

Stimulation of Klotho Expression in Aged Skin Cells with Snow Algae Extract



Result

In aged fibroblasts the activity of anti-aging gene Klotho gene was reduced.

Snow Algae extract stimulated Klotho expression and overcompensated this effect

→ Anti-aging activity

2. Activation of AMPK

Cell type

Normal human epidermal keratinocytes (NHEK)

Treatment

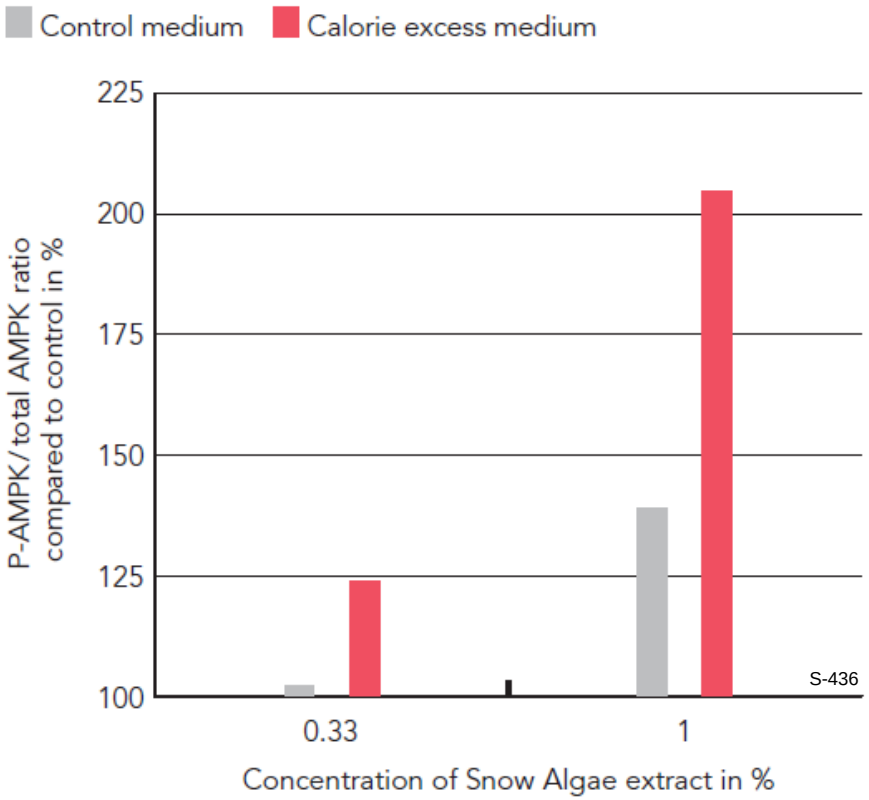
+ 0.33% and 1% Snow Algae extract

Cell cultures in normal medium or under calorie stress (60 ng/ml insulin) for 48 h

Analysis

Western Blot analysis of total AMPK (5'-adenosin-monophosphate-aktivated protein kinase) and phosphorylated AMPK (P-AMPK, active form)

Activation of AMPK



Snow Algae extract

- stimulates the phosphorylation (activation) of AMPK in a dose dependent manner
- protective effect against caloric excess induced by a high insulin concentration

→ prevents the age-related decline of AMPK

→ activation of “longevity pathway”

Stimulation of Collagen I and III in Aged Skin Cells

Cell type

Normal human dermal fibroblasts (NHDF)

Treatment

Cultivation of aged fibroblasts at passage 17

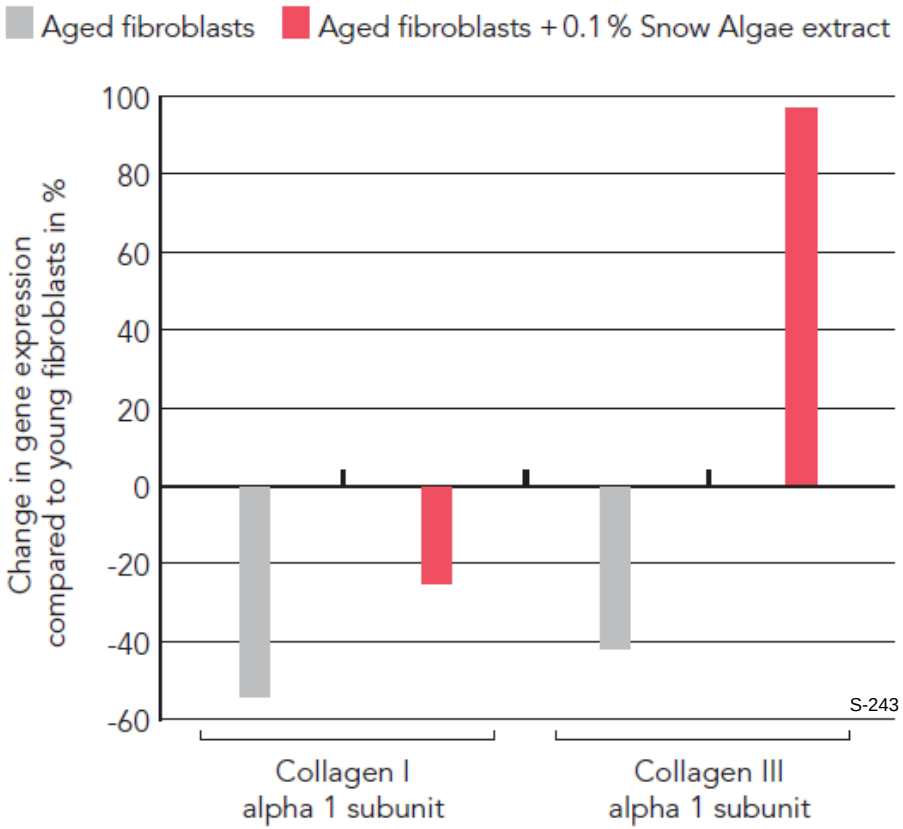
± 0.1% Snow Algae extract for 24 h

Parameter

Quantitative PCR analysis of collagen I and III

(important markers for cellular aging)

Stimulation of Collagens in Aged Skin Cells



Snow Algae extract helps to repair the effects of aging by stimulating the most abundant collagens in the skin.

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Anti-MMP Effect in Aged Skin Cells in H_2O_2-Induced Senescence Model

Cell type

Normal human dermal fibroblasts (NHDF)

Treatment

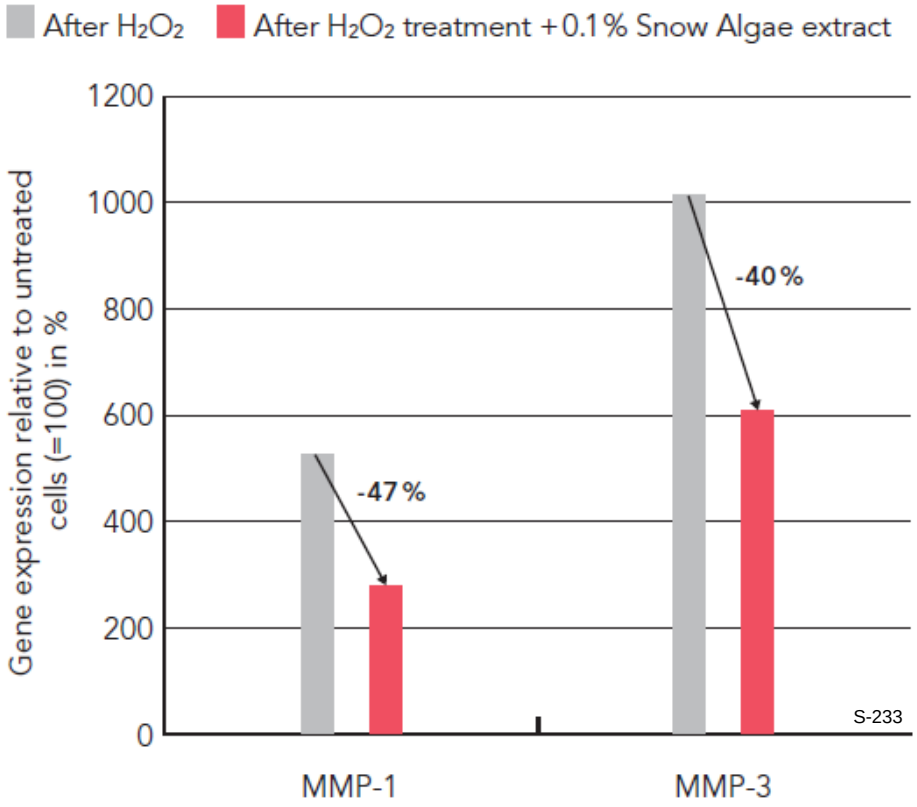
± 0.1% Snow Algae extract for 24 h

Induction of senescence with H_2O_2

Analysis

Quantitative PCR analysis of MMP genes (important markers for senescence in skin)

Anti-MMP Effect in Aged Skin Cells in H_2O_2-Induced Senescence Model



Snow Algae extract counteracts the effects of aging by reducing the MMP activity in senescent cells

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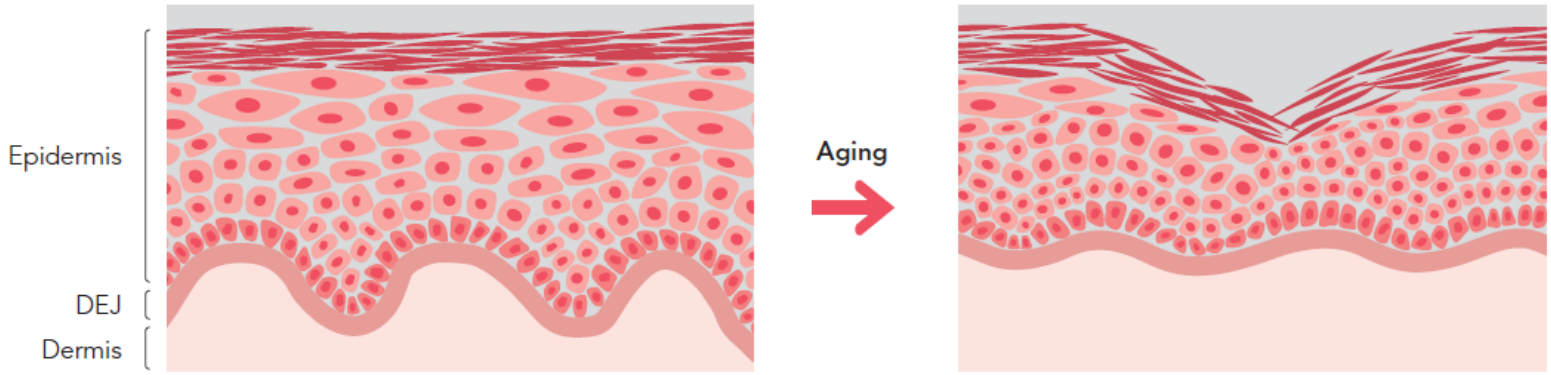
The Dermal Epidermal Junction (DEJ)



DEJ

Area that tightly binds the dermis to the epidermis. Its permeability regulates exchanges between both tissues. In addition, the DEJ contributes to the cohesion of the epidermis and to the wound healing process.

Young skin	Structure of the DEJ is wavy, maximizing the surface area of connection between the epidermis and the dermis.
Aged skin	The DEJ becomes more flattened, the connection between the epidermis and the dermis is reduced (modification in quality and quantity of elastin and collagen).

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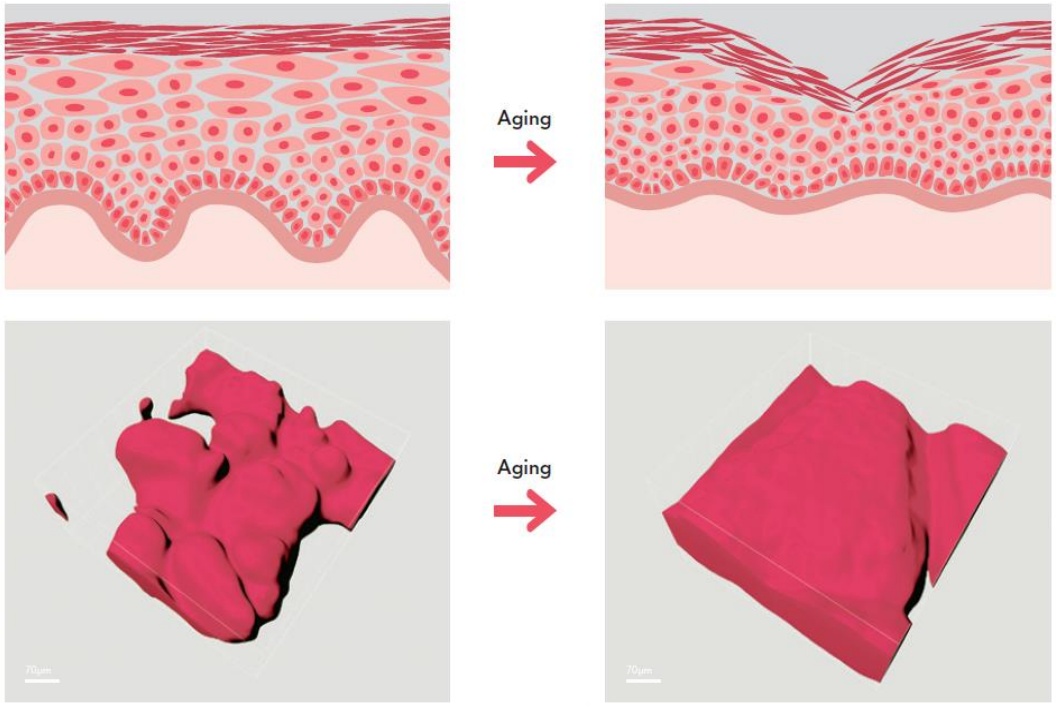
Two-Photon Microscopy to Analize The Dermal Epidermal Junction (DEJ)



"Multi-photon skin tomography images provided by Neurotar LTD (Skinvivo services, www.skinvivo.com)"

Two-Photon Microscopy

Innovative, non-invasive 3D technique to analize and visualizes deeper skin structures in-vivo.

Principle: generation of an infrared laser signal to irradiate the skin → autofluorescence of some molecules (e.g. elastin) or second harmonic generation (e.g. collagen).

Two-Photon Microscopy Study Design

Products
Hydrogel + 2 % Snow Algae Powder
Placebo

Volunteers
5 (55 - 67, f)

Application
Twice daily for 2 months
Inner side of the forearm
Placebo on the other forearm

Analysis
Dermal-epidermal junction (DEJ) surface (two-photon microscopy)

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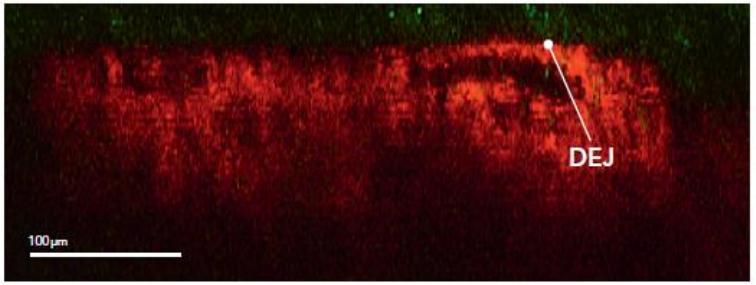
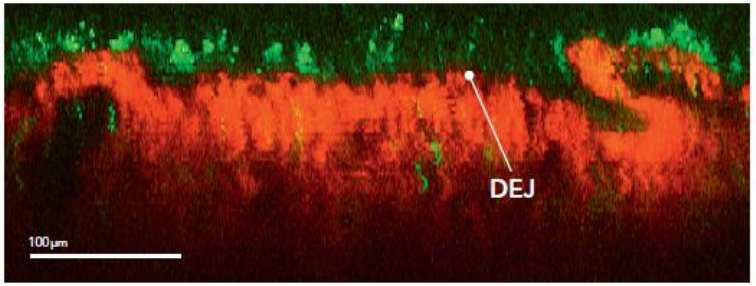


After two months of treatment with 2% Snow Algae Powder



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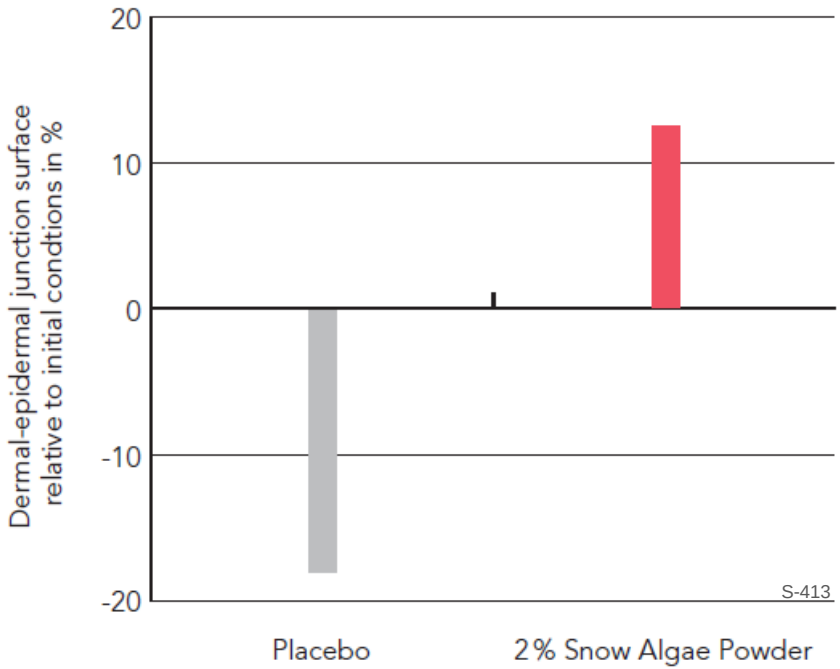
Two-Photon Microscopy
Dermal-Epidermal Junction Rejuvenation



Snow Algae Powder improved the undulations of the DEJ and increased its surface by 12.5 % compared to initial conditions and by 30.5 % compared to the placebo.

→ Rejuvenating effect of the dermal-epidermal junction

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Reinforcement of the Skin Barrier in Challenging Conditions

Double-blind study performed during winter time (alpine winter climate)

Volunteers

- 21 (30 – 57y,19 f and 2 m), phototype II

Products

- Emulsion with 3 % Snow Algae Powder, placebo

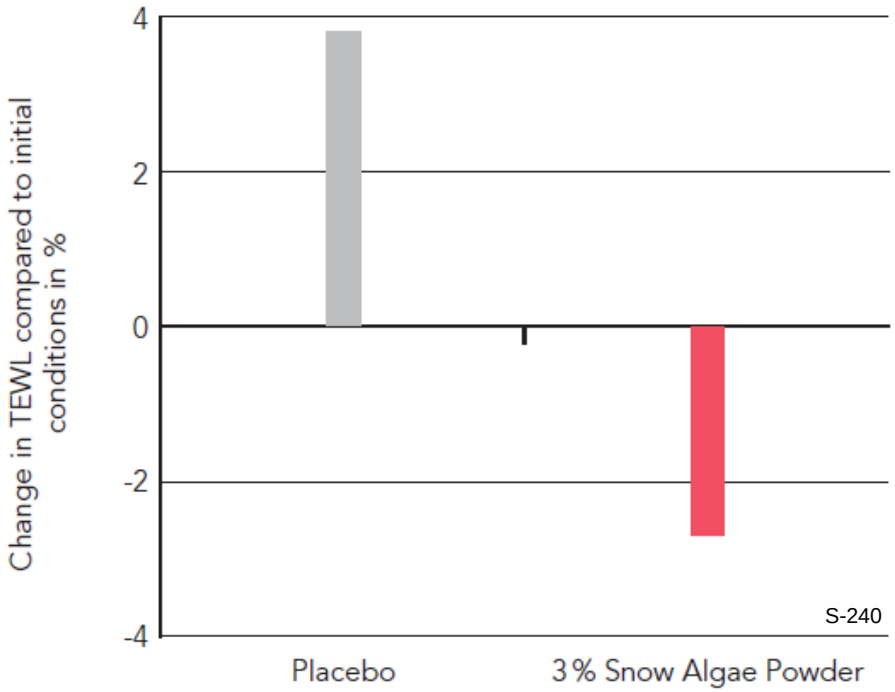
Application

- Three times daily for 21 days
- The test cream was applied on one half of the face and the corresponding placebo on the other half.

Parameters

- TEWL (cheeks): Tewameter
- Age spots: VisioFace Quick®

Reinforcement of Skin Barrier
→ Reduction of Water Loss



Winter weather conditions led to an increased loss of water by the skin (placebo).

Snow Algae Powder was shown to counteract this effect and to even reduce TEWL.

Snow Algae Powder is able to reinforce the skin barrier even under challenging conditions.

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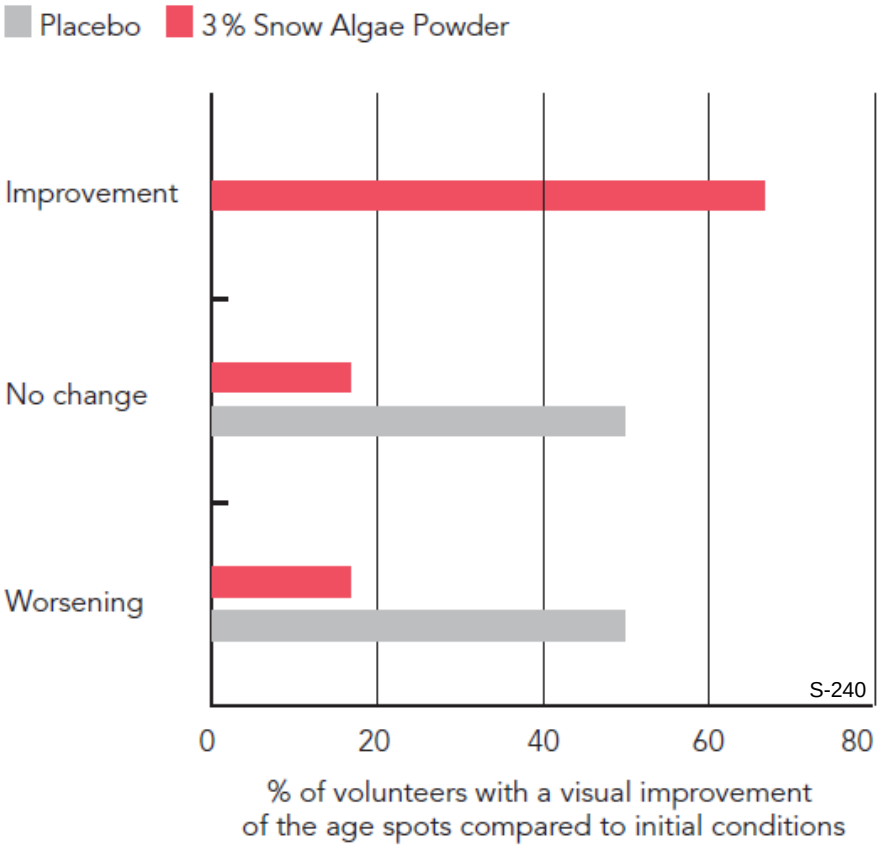
Reduction of the Visibility of Age Spots



- High definition photographs were taken using VisioFace Quick®
- Aspect of age spots was evaluated by trained assessors.
- Performed on the six volunteers presenting age spots at the beginning of the study

With Snow Algae Powder, the visibility of age spots was reduced in 67 % of the cases.

Snow Algae Powder Claim Ideas

- Protects and activates longevity factors in skin cells
- Rejuvenates and protects skin at cellular level
- Safeguards skin’s youthfulness by activating Klotho
- Strengthens cellular defense mechanisms through calorie restriction mimetic activity

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Snow Algae Powder Applications

- Rejuvenating and repair formulas
- Age-defense products
- Youth protecting and promoting skin care
- Formulas to increase skin's longevity – face, body, hand

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Snow Algae Powder Marketing Benefits

- Unique extremophile algae
- Sustainable production by biotechnology
- Novel anti-aging concept by Calorie Restriction Mimetic Activity
- Free logo to label your products that contain Snow Algae Powder; as a quality label
- Preservative-free
- Patent (US 8,206,721 B2)