

Stimulation of Klotho and AMPK activity to mimic caloric restriction-induced anti-aging activity in skin

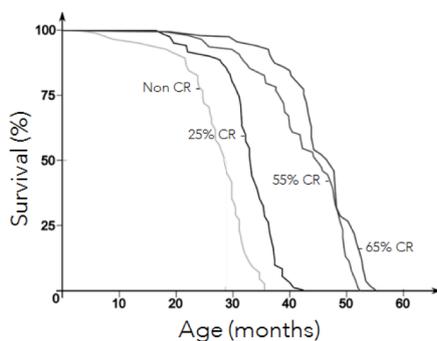
Fred Züllig Ph.D., Daniel Schmid Ph.D.
Mibelle Biochemistry, 5033 Buchs, Switzerland

Abstract

The concept of caloric restriction to retard signs of aging and to extend lifespan has gained a lot of interest in the past years. In our work we show for the first time caloric restriction-mimetic activity as a new anti-aging strategy in cosmetics. Up-regulation of Klotho expression and stimulation of AMPK activity was used to activate FOXO, the central cellular switch in the caloric restriction-induced longevity phenomenon. In cell culture assays, a *Chlamydocapsa* algae extract was found to strongly enhance the expression of Klotho and to stimulate activation of AMPK. Anti-aging effects on skin could be demonstrated in a series of clinical studies.

Introduction

Caloric restriction is the most recognized mechanism to retard signs of aging and to extend lifespan.



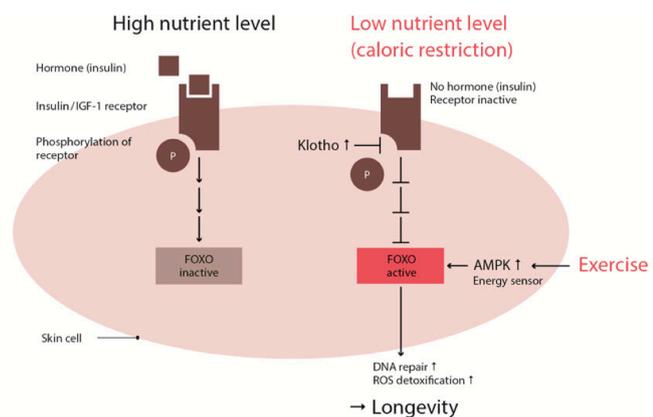
Caloric restriction (CR) increases lifespan in mice. Data taken from: Weindruch R et al. (1986), *Journal of Nutrition* 116(4), 641-54.

Studies in yeast, mice, rats and apes proved that the reduction of the daily caloric intake by 30% with optimal nutrition significantly extended life expectation and improved health. This concept has gained a lot of interest in the past years and a lot of research was conducted to study the biochemical mechanisms behind the phenomenon. Of utmost interest in this context is the research of drugs which would lead to these anti-aging effects without the hassle of dieting.

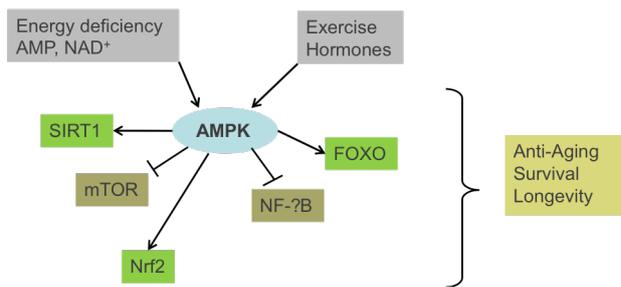
The longevity response of caloric restriction is regulated by nutrient and energy sensing pathways. The insulin/IGF-1 signaling pathway is a cellular sensor for nutrients. Under high nutrient and insulin conditions, the receptor gets phosphorylated, leading to inactivation of the Forkhead transcription factor FOXO inside the cell. Under low-nutrient conditions, this signaling 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.

Suppression of the insulin/IGF-1 signaling pathway is regarded as the central mechanism in the calorie restriction-induced longevity phenomenon. 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 aging. AMPK can activate FOXO and Nrf2, another activator of the skin's own defense systems. AMPK blocks NF- κ B and thus inhibits inflammatory reactions. Stimulation of AMPK activity induces anti-aging effects and confers longevity.



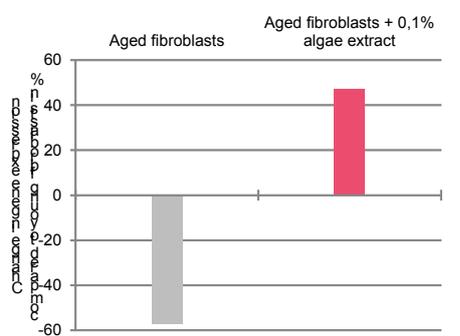
Another important factor in aging is the Klotho gene. Klotho (-/-) mice display an accelerated aging phenotype including skin appearance and on the other side when overexpressed, the gene extends lifespan by 30%. There are two forms of the Klotho protein known: transmembrane Klotho acting as co-receptor of fibroblast growth factor 23 and secreted Klotho acting as hormone. The latter was found to mediate its effects by inhibiting the insulin/IGF-1 signaling at the level of the insulin receptor substrate protein. The secreted Klotho protein activates thus a longevity response mediated by a caloric restriction pathway.



In our work here, we report for the first time on the application of this concept to delay skin aging. The strategy was to screen natural materials for activation of Klotho expression and/or stimulation of AMPK activity to induce longevity effects independent of the nutrient and energy status.

Results and Discussion

Extracts of a series of extremophilic microorganisms were analyzed for stimulation of Klotho gene expression in human primary fibroblast cultures. In a replicative aging model, cells of passage 17 were found to express Klotho gene at a ratio of 0.43 compared to cells of passage 8. We found that an algae extract of a *Chlamydocapsa* species was able to increase the Klotho expression in aged cells 4.9 fold compared to control passage 17 cells. In the same experiment, the treatment with the algae



Stimulation of Klotho expression in aged cells

extract was found to up-regulate also the expression of collagen I and III in aged fibroblasts by 63% and 240% relatively compared to untreated passage 17 cells.

Stimulation of Klotho should induce activation of the FOXO transcription factor. This leads ultimately to the activation of the cellular defense systems and confers oxidative stress resistance. In accordance with this, the algae extract was found in a H_2O_2 -induced oxidative stress model with human primary fibroblasts to significantly reduce the over-expression of matrix metalloproteinases I and III, by 47 and 40% respectively. The effect of the algae extract on phosphorylation of AMPK was tested on human primary

keratinocytes under normal conditions and in presence of high insulin concentrations representing high nutrient

conditions. The algae extract was found to stimulate phosphorylation of AMPK, especially under high nutrient conditions, by 105% in that case. The cell culture assays done so far show that the algae extract promotes transcriptional activity of FOXO by stimulating Klotho and AMPK. This will lead to upregulation of cellular defense systems and resistance to oxidative stress.

In clinical studies, the algae extract was found to increase hydration, to reduce TEWL and to improve cutaneous micro-relief. Two-photon microscopy was used as a novel non-invasive method to analyze 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. A cream with the extract was applied by 5 women in the age of 55 – 67 on the inner side of the forearm during two months. The placebo cream was applied on 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 placebo. A rejuvenation effect was also shown by the placebo-controlled increase in the collagen to elastin ratio (+12%).

Conclusion

To conclude, we propose for the first time caloric restriction-mimetic activity as a new anti-aging strategy in cosmetics. With our studies we could already identify an extract of an extremophile alga which shows positive results in this new approach. The activation of Klotho and AMPK leading to an upregulation of FOXO-regulated genes in skin cells will promote cellular defense systems and confer oxidative stress resistance. Thus caloric restriction-mimetic activity by active ingredients may become a new strategy for anti-aging cosmetics.