

Proteins that have a relatively low turnover rate, such as collagen and elastin, can be especially sensitive to non-enzymatic protein glycation. One type of glycation, a glucose modification of amines, is removed by fructosamine-3-kinase (F3K), a novel enzyme that phosphorylates fructosyllysine. Although the non-modified amino acid is ultimately restored by this process, a byproduct of the reaction is 3-deoxyglucosone (3DG), a highly reactive and toxic molecule. 3DG mediates numerous downstream effects including inflammation, oxidative stress and formation of advanced glycation endproducts (AGEs). F3K and its toxic byproduct 3DG are present in skin, and the effects of 3DG can contribute to skin aging. Research based upon these findings has led to the development of topical skin care products that target 3DG production and have statistically significant anti-aging and anti-inflammatory effects in blinded human trials.

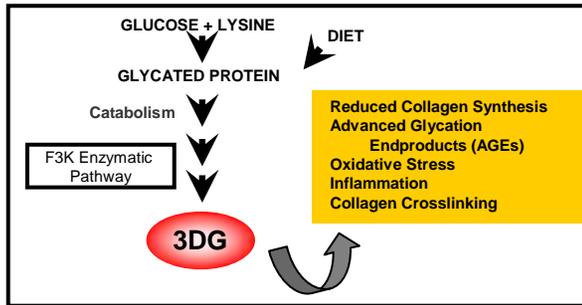
The use of topical preparations that contain an F3K inhibitor (sugar analog) and a 3DG inactivator (amino acid) caused an improvement in the appearance and moisturization of aging skin in clinical trials. This ingredient combination was used in a study with 18 volunteers showing photo-damaged skin on the upper arm. After 4 weeks of twice daily use, there were statistically significant improvements in an expert grader's assessments of visual dryness and texture (loss of crepiness) compared to use of a base cream. A separate test with 75 volunteers with photo-aged facial skin showed a statistically significant, quantitative decrease in fine facial lines (replica analysis), an increase in moisturization and an increase in firmness after 2 weeks of twice daily application of a topical preparation containing the F3K inhibitor and 3DG inactivator. There were further improvements after an additional 4 weeks of treatment. In a wound healing trial, preparations of a base cream and a base cream containing an F3K inhibitor and a 3DG inactivator were tested in a single-blind study with 12 volunteers. Six sites on the volar forearms (3 on each arm) were exposed to an irritant solution (0.5% sodium lauryl sulfate) under occlusion. After 18-24 hr, the patches were removed and panelists then had the test creams applied to four sites twice daily for 7 days. The other forearm sites were untreated and used as controls. Extent of irritation and healing rates were based on the observations of an Expert Grader and measurements for TEWL and redness on day 0 (prior to SLS exposure), and subsequent days. Skin treated with the F3K inhibitor and 3DG inactivator showed decreased redness, and increased healing, with the greatest benefit seen after 3-8 days. Taken together, these results indicate that the 3DG pathway is a viable target for anti-aging skin care.

# INHIBITORS OF 3-DEOXYGLUCOSONE TREAT AGING AND INFLAMED SKIN BY PREVENTING THE FORMATION OF AGES, OXIDATIVE STRESS AND FREE RADICALS

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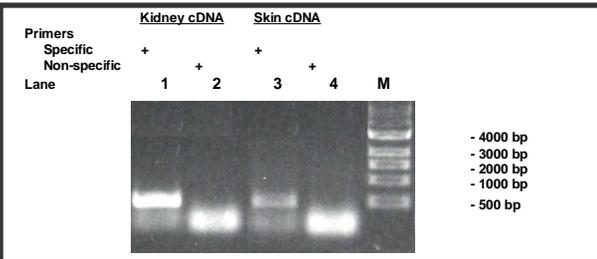
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## I. Protein Glycation and 3DG Pathway

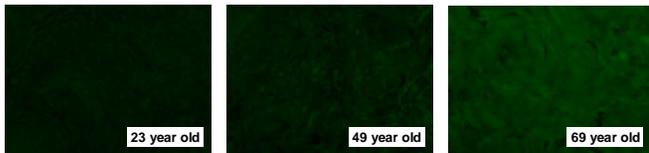


The biochemical pathway shows the production of 3-deoxyglucosone (3DG), a metabolite derived from sugar (from direct ingestion or catabolism of glycated protein and high fructose corn syrup). 3DG is a highly reactive dialdehyde that causes inflammation, oxidative stress, free radicals, collagen crosslinking, and the formation of detrimental advanced glycation endproducts (AGEs). 3DG targeted cosmetic ingredients decrease the conversion of glycated protein to 3DG and inactivate 3DG.

## II. Age-dependent Glycation Damage to Skin



Enzymatic glycation pathway to produce 3DG is in skin. Poly A+ messenger RNA from human kidney and skin was used in a RT-PCR reaction with either F3K specific (lanes 1 and 3) or non-specific (lanes 2 and 4) oligonucleotide primers. The only DNA bands are those due to the presence of the F3K-specific primer.

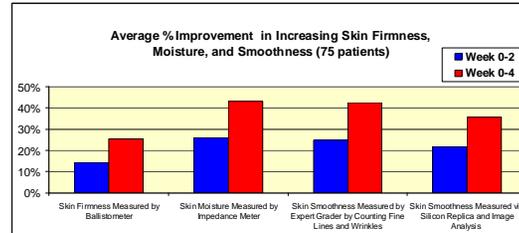


Photos provided by Dr. Carol Artlett, Thomas Jefferson University

Effect of 3DG in skin. 3DG reacts with proteins to form the covalent adduct imidazolone. Thin sections of skin were reacted with a fluorescent antibody to imidazolone. The amount of 3DG adduct increases with age.

## III. Clinical Effects-Anti-wrinkling

**Facial Wrinkle Trial Design:** A cream containing the 3DG-targeted active components was tested for the ability to improve skin smoothness, texture and moisturization after a 4 week treatment. A group of 75 volunteers applied the cream twice daily to the face for 4 weeks. The moisture content, firmness and wrinkles (analyzed by expert grader and silicon replica) were measured at the beginning of the study, after 2 weeks and after the 4 week period. **Results:** The graph below shows the statistically significant improvement for all features. The improvement in all parameters continued for a further 4 weeks (data available upon request).



Appearance of trial volunteer with facial lines before and after 4 weeks of twice daily treatment with cream containing 3DG inhibitors. Photos were taken by a contract clinical research study group and have not been retouched.



**Crepey Skin Arm Trial:** 18 human volunteers used either a base cream or a base cream containing 3DG targeted actives that were applied twice a day to photo-damaged, wrinkled areas of the upper arm. Photos were taken at study initiation (week 0) and again after 4 weeks of treatment.



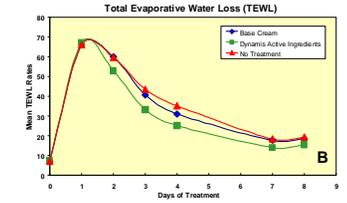
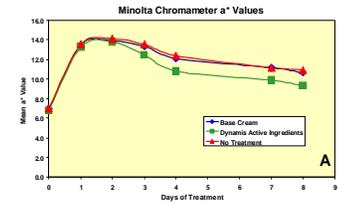
## III. Clinical Effects (cont'd)-Anti-inflammatory

### SLS Wound Healing Trial Study Design:

This was a single-blind, randomized wound healing study comparing a base cream containing the 3DG-targeting actives and the base cream alone performed by an independent research group. Six sites on the volar forearms (3 on each arm) of 15 female volunteers were exposed on Day 0 to an irritant solution (0.5% sodium lauryl sulfate) under occlusion for 18-24 hr. On Day 1, the four arm sites with the most similar degree of damage for 12 of the volunteers who experienced a significant irritation effect of the SLS were selected for the treatment phase of the study. Patches were removed and panelists then had the test creams applied to the four selected sites twice daily for 7 days. The other forearm sites were not treated so they could be used as controls.

### Results:

Figures A and B show the extent of irritation and healing rates based on measurements using a Minolta Chromameter (measures redness) and DermaLab Meter (measures Total Evaporative Water Loss (TEWL)) on day 0 (prior to SLS exposure), and on days 1, 2, 3, 4, 7, and 8. From Days 2-4 there were significant differences between the cream containing the 3DG targeted actives and the base cream or non-treated controls. The cream with the 3DG targeted actives is most effective at reducing erythema and water loss. Figure C is a photograph of the SLS-treated areas after 8 days of treatment.



## IV. Summary

- ♦ Glycation produces a highly reactive dialdehyde called 3-deoxyglucosone (3DG) that can damage skin proteins. This pathway is present in skin and the extent of damage to skin proteins increases with age.
- ♦ A facial cream with novel active components that target 3-deoxyglucosone (3DG) has benefits for the treatment of aging, wrinkled and photo-damaged skin. Twice daily application results in skin that is smoother, firmer, and more moisturized after 2 weeks application.
- ♦ This preparation also has benefits for the treatment of detergent damaged skin. Twice daily application results in decreased redness and water loss and faster healing.

**Disclosure:** Authors are employees of Dynamis Skin Science and studies were funded by Dynamis Skin Science.