# Energizing the skin with phytoglycogen

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Living organisms require energy to perform the tasks of everyday life. At the cellular level, the energy is used for overall cell maintenance, protection, and the production of molecules that are essential for cell and organ function.

Skin cells, which are continuously exposed to environmental factors such as sunlight or pollution, greatly rely on large amounts of energy to combat cellular – and consequently skin – damage. As we age, cellular energy levels decline and the deficiencies in skin energy can manifest in age-related changes in the skin.[1]

One consequence of this includes the formation of wrinkles or skin hyperpigmentation due to photodamage. Therefore, increasing cell energy levels can be a valid approach to target skin ageing.

To maintain a healthy and youthful skin, the skin produces various essential molecules including hyaluronic acid (HA) and collagen (Figure 1). The production of these molecules requires energy, levels of which are unfortunately reduced during the ageing process.

Indeed, decreased levels of HA and collagen are also observed in the ageing skin.[2] Evidently, increasing the available energy necessary to boost the production of these molecules can benefit the skin.

A preferred energy substrate for cell metabolism is glucose, which is broken down via a series of biochemical reactions to release energy in the form of ATP (adenosine triphosphate). Glucose can be packed and stored for later use.

This ensures sufficient energy sources are available, even in times of stress. Efficient and dense packaging is required to store as much glucose as possible while using minimal space. One of the densest storage units of glucose in the cell is glycogen.

## Glycogen: Densely packed energy for skin rejuvenation

Glycogen is an energy-storage unit found in mammalian cells. It is a highly branched structure of repeating glucose units that are linked together by specific bonds, termed α(1-4) and α(1-6) glycosidic bonds. The structure of glycogen itself is very dense, making this molecule an ideal storage unit of energy inside the cell (Figure 2).

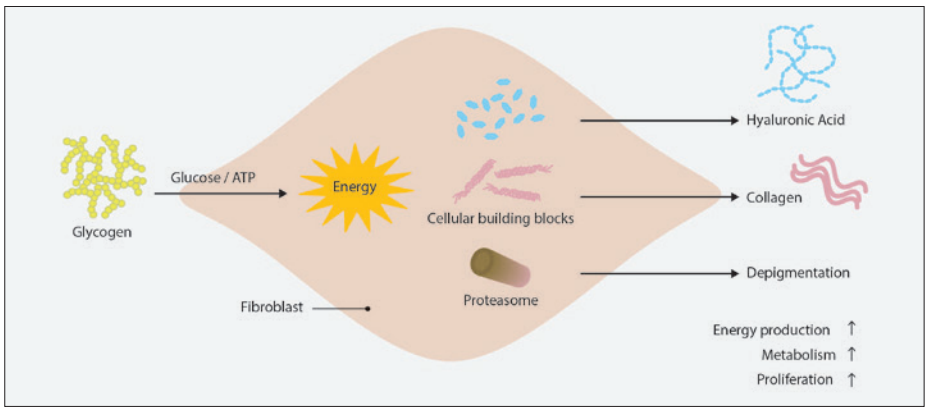
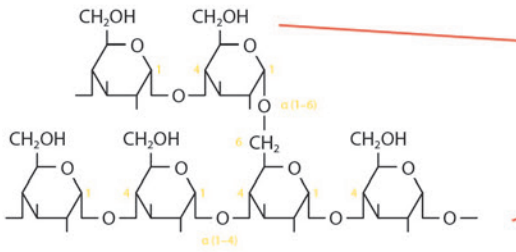



**Figure 1:** Skin cells, energy and metabolism

**ABSTRACT**

Skin cells are continuously exposed to environmental stresses, which makes them greatly reliant on large amounts of energy to combat cellular – and consequently skin – damage. As we age, cellular energy levels decline and the deficiencies in skin energy can result in premature skin ageing. By providing glycogen, the cells are given energy to use whenever it is needed and wherever it is needed, resulting in many beneficial effects for the skin. However, glycogen is usually isolated from animal sources. In this article a new plant-based glycogen is described, isolated from a special non-GMO sweetcorn, which is chemically identical to glycogen stored in animal cells. *In vitro*, the phytoglycogen was able to stimulate ATP production and cell metabolism, resulting in increased hyaluronic acid and collagen production. Clinically the topical application of the phytoglycogen provided fast and long-lasting hydration effects, led to anti-ageing benefits and boosted the effects of hyaluronic acid. In summary, the safe and natural phytoglycogen is a multi-purpose cosmetic active, yielding excellent benefits for the skin.




| | Glycogen | Phytoglycogen |
|---|---|---|
| Source | Animal | Plant (special sweet corn variety, non-GMO) |
| Function | Energy storage | Energy storage |
| Subunit | α-glucose | α-glucose |
| Bonds | 1–4 and 1–6 | 1–4 and 1–6 |
| Branches | Yes (~per 10 subunits) | Yes (~per 10 subunits) |

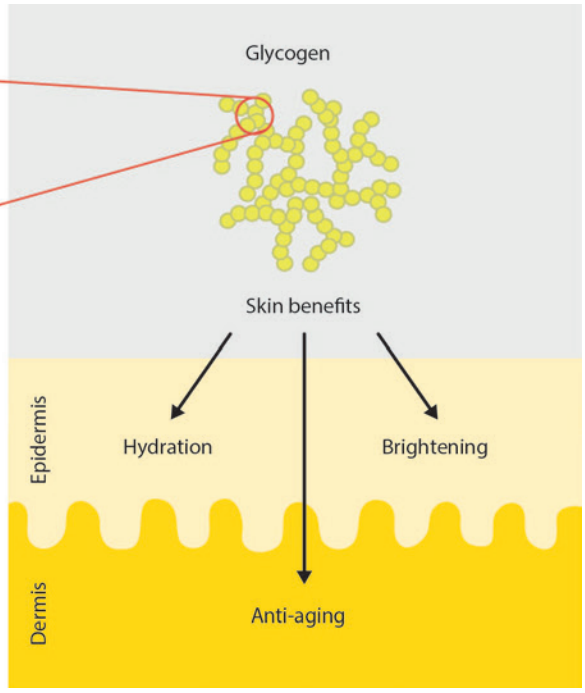



**Figure 2:** Plant-based glycogen: Structure and skin benefits

The largest stores of glycogen are usually found in the muscles and liver, which are very metabolically active and require large amounts of energy. However, a small quantity of glycogen is also present in the skin, where it is an important skin energy source.[3]

Importantly, glycogen content in the skin also declines with ageing, which suggests that glycogen loss may also play a role in skin ageing. By providing glycogen, the skin cells are given more energy not only to produce essential molecules,[4] but also to remove damaged molecules.[5]

Delivering energy to improve the skin damage response, such as the degradation of damaged molecules by the proteasome, can consequently help prevent skin hyperpigmentation.[6] As such, glycogen may have many benefits for the (ageing) skin and is, therefore, a popular ingredient for cosmetic formulations.

There is, however, one problem: Glycogen is the glucose storage unit found in mammalian cells and it is usually isolated from animal sources. To circumvent this, an alternative vegan solution has been developed: PhytoSpherix™, a plant-based phytoglycogen.

## A natural and safe plant-based glycogen to energize the skin

The plant-based glycogen is naturally produced by a special variety of non-GMO sweetcorn and extracted via a mild water-based process. Various physical characterization techniques have investigated and confirmed the structure to be chemically identical to that of mammalian glycogen,[7,8] but it is produced and stored in plants.

It is a compact, spherical, and water-misceble molecule, whose highly branched dendrimer structure further yields some interesting properties, including high water-binding, low viscosity, and high stability in water. The efficient water-binding capacity, for example, may enhance the moisturizing properties of skincare formulations.[9]

*In vitro* and clinical studies have demonstrated that the phytoglycogen has rapid and long-lasting hydrating effects, further reduces skin hyperpigmentation and elicits anti-ageing effects. In summary, the multipurpose active is ideal for cosmetic formulations, as a natural and safe, plant-based alternative to animal-derived glycogen.

## Methods

### Cell culture and treatment

For the ATP quantification, normal human epidermal keratinocytes (NHEK) were seeded to 96-well plates and cultured in keratinocyte culture media (serum-free, supplemented with growth factors) for 24 hours, at 37°C and 5% $CO_2$.

The medium was then replaced with assay medium (serum-free, no growth factors, 1g/L glucose) containing or not (control) 1% phytoglycogen for two hours and 24 hours.

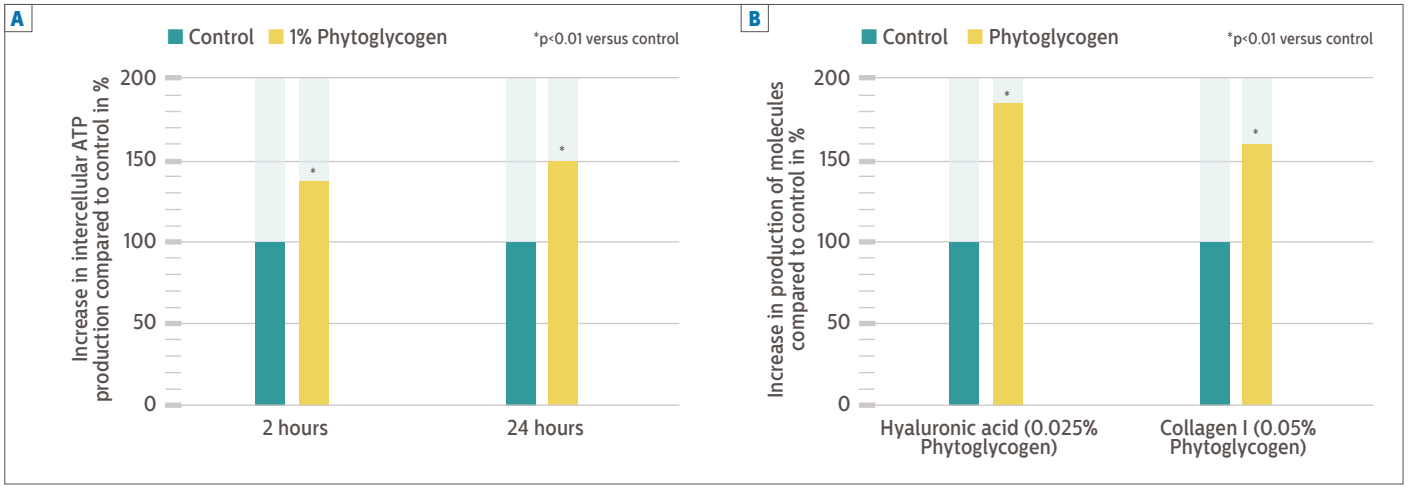



**Figure 3:** Stimulating effects on cellular ATP, HA and collagen I production

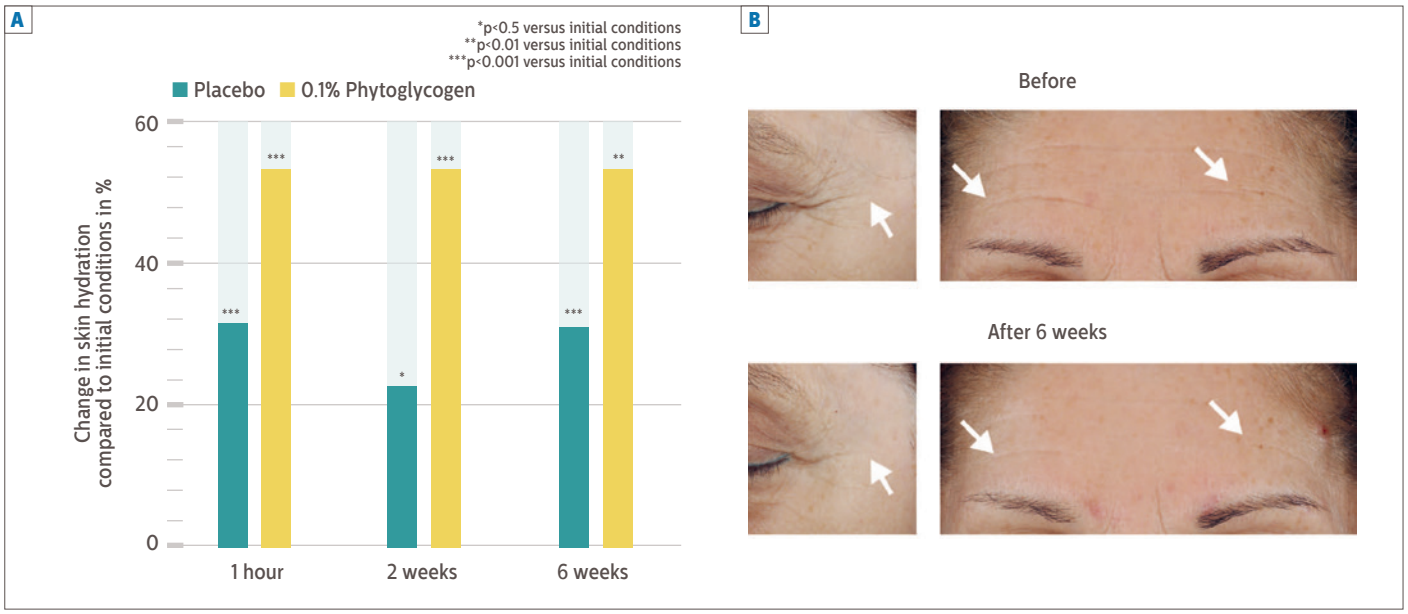



**Figure 4:** Clinical: Hydrating and anti-ageing effects of the phytoglycogen

For the quantification of HA and collagen I, human foreskin fibroblasts (ATCC, SCRC-1041, USA) were cultured in 75 $cm^3$ tissue culture flasks in DMEM containing 15% FBS at 37°C and 5% $CO_2$. During treatment, all cells were cultured in DMEM containing reduced glucose (0.2g/L).

For the HA measurements, fibroblasts were grown in 96-well plates and treated or not (control) with 0.025% phytoglycogen for 36 hours. For collagen I evaluation, the fibroblasts were grown on glass coverslips in 12-well plates and treated or not (control) with 0.05% phytoglycogen for 36 hours. All experiments were performed in n=3.

### Quantification of intracellular ATP

After treatment, the intracellular ATP content of the NHEK was measured using a GloCellTiter-Glo luminescent cell assay (G7571, Promega, USA) according to the manufacturer's instructions. Briefly, equal volumes assay reagent were added to the cell layers, the plates then placed on an orbital shaker for two minutes to induce cell lysis.

After ten minutes stabilization at room temperature, the luminescent signal was detected on a fluorescent microplate reader (Perkin Elmer). A standard curve was established using rATP (P1132, Promega, USA) and detected ATP was normalised to total protein content of the cells.

### HA and collagen I content

For the HA quantification, the media supernatant of the fibroblasts was collected after treatment and the amount of HA present in the media measured and quantified using an enzyme-linked immunosorbent assay (ELISA), as per the manufacturer's instructions. For the collagen production, the cells were washed and fixed with a formalin solution before being processed for immunohistochemistry.

Collagen content in the cell was labeled and visualized with a primary antibody against human type I collagen, and a fluorescent secondary antibody. Cell nuclei were stained with DAPI. Images were taken using a fluorescent microscope (Nikon Ti Eclipse, Nikon, Japan) and the fluorescence intensity quantified.

### Clinical studies

To evaluate the clinical efficacy of the phytoglycogen, two double blinded randomized and placebo-controlled studies were performed. In the first study, the hydrating and anti-ageing effects were evaluated on 63 female volunteers aged 51 – 65 years with moderate global facial photodamage and self-perceived dry skin.

The volunteers applied a cream containing 0.1% phytoglycogen to the whole face, twice daily, for six weeks. The measured parameters were skin hydration (Corneometer CM 825, Courage + Khazaka, Germany), skin hyperpigmentation (expert clinical grading) and skin even tone (expert clinical grading).

Images from select volunteers were acquired with VISIA CR2 (Canfield Imaging Systems, USA) and the forehead wrinkles, crow's feet and pigment spots analysed further with Newtone Inc. proprietary technology (Newtone Inc., USA).

For the pigment spot analysis, the L*, b* and a* factors of the skin inside the pigment spot and the skin surrounding the spot were measured an, yielding a difference in contrast (visibility) of the pigment spot.

In the second study, the moisturizing properties of the phytoglycogen was further investigated. Thirteen healthy male and female volunteers aged 25 – 60 years were recruited, and different formulations were tested, containing the phytoglycogen and/or HA (MW 800-1200 kDa, MakingCosmetics, USA).

The tested formulations were either a cream containing 0.1% phytoglycogen, a cream containing 0.1% HA, or a cream containing 0.05% phytoglycogen and 0.05% HA combined. Each volunteer applied all three formulations at defined areas of the inner forearm, a fourth area was added for untreated (no formulation) control.

After a single application, skin hydration measurements (Corneometer CM 825, Courage + Khazaka, Germany) were taken over a time course of seven hours and compared to baseline and untreated controls.

## Results and discussion

The effects of the phytoglycogen on intracellular ATP production was firstly investigated *in vitro* in keratinocytes. As one of the main energy-carrying molecules found in the cell, ATP drives many important processes, including metabolism, proliferation and the production of essential molecules.

After just two hours, treatment with 1% phytoglycogen resulted in a significant stimulation of ATP production (Figure 3A). The levels were further increased after 24 hours by almost 50% compared to untreated controls.

Positive effects of the phytoglycogen were also evident in human fibroblasts. Treatment with 0.025% led to a significant increase in HA secretion from the fibroblasts. Compared to control, HA secretion was increased by 83% (Figure 3B).

Furthermore, treatment with 0.05 % phytoglycogen significantly induced collagen I. In conclusion, the phytoglycogen can induce ATP levels, thereby stimulate cell metabolic activity, which results in the increased production of beneficial molecules such as HA and collagen.

The increased HA levels, as observed in the *in vitro* assays, can have beneficial effects such as better skin hydration. This was investigated in a clinical study, where the volunteers applied a cream containing 0.1% phytoglycogen over a total of six weeks.

Just one hour after the first application, the treatment significantly increased skin hydration by 53.3% compared to baseline, which demonstrates the immediate efficacy of the plant glycogen.

This hydration increase remained significantly enhanced after two weeks and six weeks of treatment. Furthermore, after six

weeks of treatment a pronounced decrease in crow's feet wrinkle area, length and volume was observed.

Compared to initial conditions, crow's feet wrinkle area and volume were decreased by 42% and 49.6% respectively. Furthermore, the wrinkle length was decreased by 29.6% compared to initial conditions.

Similar effects were observed when analysing the forehead wrinkles. Compared to initial conditions, forehead wrinkle area and volume were decreased by 54.9% and 45.6%, respectively. The overall forehead wrinkle length was decreased by 53.2%.

Taken together, treatment with phytoglycogen results in beneficial effects for the skin, including a better skin hydration and a reduction of wrinkles. This ultimately yields a more even and healthy-looking skin.

Glycogen can deliver energy, which may aid in the skin damage response and possibly help prevent skin hyperpigmentation. Previous *in vitro* studies revealed a minor but significant inhibition of melanin synthesis with 0.5% phytoglycogen.

Therefore, the clinical effects of phytoglycogen on skin hyperpigmentation was investigated. After treatment with 0.1% phytoglycogen for six weeks, skin hyperpigmentation and skin tone evenness were significantly improved, by 30% and 37%, respectively (Figure 5A).

This reduction in skin hyperpigmentation was also evident in the analysis of the pigment spots. After six weeks, the contrast differences of the spots improved by 22.1% for L* 24.1% for a* 15.2% for b* and they became less visible (Figure 5B). Therefore, the skin-energizing effects of the phytoglycogen can also improve skin hyperpigmentation, leading to a more even complexion.

As previously shown, treatment with the phytoglycogen improved skin hydration. A known benchmark for skin hydration includes the use of HA in formulations. However, this can encounter certain difficulties including viscosity issues of the formulation.

Therefore, exploring effective alternatives to HA are of increasing interest. The moisturizing effects of the phytoglycogen, compared to or combined with HA, were investigated with surprising results.

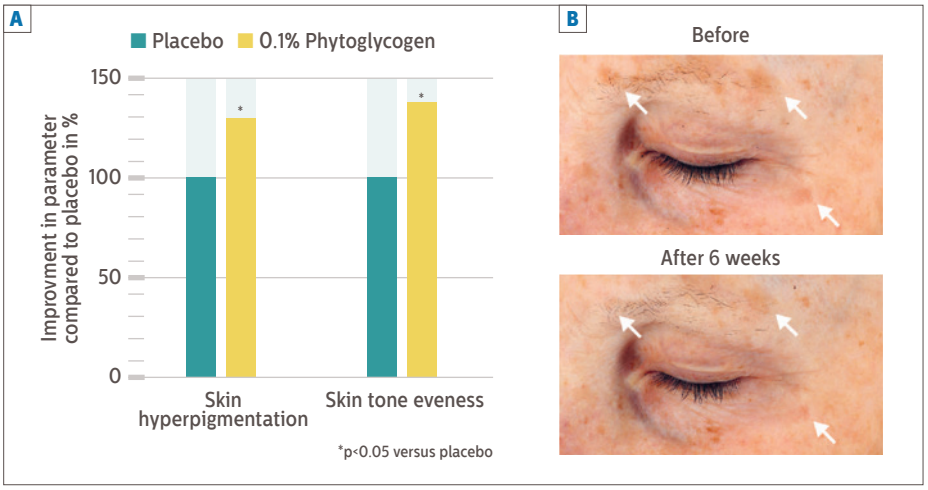



**Figure 5:** Clinical: Reduction of skin hyperpigmentation

After just one hour, a single treatment with 0.1% phytoglycogen boosted skin hydration by 155.3%, compared to baseline, demonstrating again the immediate hydrating effects. This effect was still present after seven hours, which further highlights a long-lasting effect on skin hydration. Importantly, these effects were comparable to the treatments with 0.1% HA (Figure 6).

Intriguingly, the combination blend with lower concentrations (0.05% each) of phytoglycogen and HA led to a significantly higher hydrating effect (+199% after just one hour). This indicates a synergistic effect of the two actives, leading to superior effects to the treatments with the single actives alone.

Evidently, formulating with the plant-based glycogen can boost the skin moisturizing effects of HA. Therefore, less amounts of either ingredient are required to achieve the same effect, and HA formulations can reach higher hydration levels without sacrificing texture or skin feel.

## Conclusion

The novel plant-based glycogen, isolated from a special non-GMO sweetcorn, has numerous benefits for the skin. By energizing the skin cells, it can boost cell metabolism and promote HA and collagen I production. This results in anti-ageing benefits, including a reduction of wrinkles and pigment spots.

Furthermore, the immediate and long-lasting hydrating effect make the phytoglycogen an excellent ingredient for cosmetic formulations. Moreover, the active has HA-boosting effects, which allows formulators to reduce the amount of HA used, without sacrificing moisturizing effects.

In summary, the phytoglycogen is a safe and natural multi-purpose cosmetic active, which yields excellent benefits for the skin. **PC**

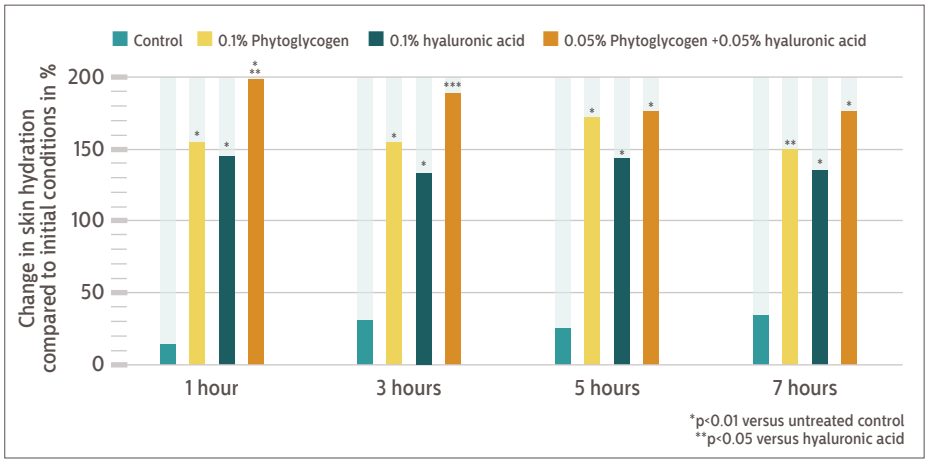



**Figure 6:** Synergistic hydration effect with hyaluronic acid

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