

Energise your skin for a glow of youth

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The modern lifestyle is an exhaustive lifestyle: augmented fitness and leisure activities, intensified human relations, accelerated communication, tiring business... However, in these times of multi-tasking, our body not only needs to function, but should be energy-filled and powerful. The same applies to our skin: in periods of exhaustive lifestyle, the skin not only needs to tolerate the stress, but should have a radiant and youthful glow.

The solution of this energy crisis is not easy, because it is not about slowing down our lifestyle! It is more about how we and our skin deal with busy times and falling energy levels. It is about keeping pace, healthiness and radiance by boosting the skin's energy supply!

In other words, consumers want to complement this change in lifestyle. Consequently, they have a strong desire for energising, recharging and youth-activating cosmetic ingredients. This fact explains that the trend 'Powerplay', which is represented by energy claims, is one of the key trends that impact the beauty and personal care industry over the next decade (revealed by Mintel!). This trend drives the cosmetic market towards a variety of appealing concepts, such as ATP boosters, repower ingredients, fatigue-fighting products, youth-activating rechargers, and many more to come.

Mitochondria produce energy but cause collateral ROS emission

Mitochondria are cellular organelles that produce about 90% of the organism's energy. Each cell, depending on its metabolic demand, contains about 100–2,000 mitochondria. From a technical point of view, energy production in mitochondria is a combustion reaction that yields ATP, the energy currency of life. ATP functions as a biological fuel for all kind of cellular activities and can be considered a universal source of energy.²

The process of energy production in mitochondria is not completely error-free and leads to the generation of reactive oxygen species (ROS). ROS firstly attack the mitochondria itself and thereby initiate a

Abstract

In times of exhaustive lifestyles, we need to compensate the skin's high energy demand in order to keep skin's healthiness and radiance. Active, modern and conscious consumers thus strongly seek for energising and recharging cosmetic ingredients to maintain a radiant skin perfection with a glow of youth, even in periods of high intensity.

HerbaGlow® NRG unites extracts of caper flower buds, mulberry leaves and rose roots in a preservative-free and self-preserving solvent system. The COSMOS-approved anti-ageing active targets the number and quality of mitochondria, which are the energy powerhouses of the cell. An extra boost of energy recharges tired-looking skin, yielding a fresh-looking, healthy and youthful glow.

This makes the active ingredient a perfect fit for cosmetic concepts such as energy-centric formulations, skin burnout treatments, and youth-activating as well as applications aiming to improve skin glow and radiance.

harmful feedback loop that steadily increases collateral ROS emission and gradually diminishes mitochondrial energy production.

Indeed, as we age, mitochondrial ATP production and overall cellular energy levels decline and production of toxic ROS increases.^{3,5} However, it is not only age, but also a demanding lifestyle that can cause energy crisis and oxidative stress, because increased mitochondrial energy production is accompanied by increased ROS production. This cumulated oxidative damage of tissue cells eventually drives premature ageing. Taken together, mitochondria are the major source and target of ROS, and thus these organelles play a key role in the ageing process,^{1,6} including skin fatigue and ageing.

In contrast, vital skin cells contain high numbers of healthy mitochondria with elevated energy production rates and low ROS emission. This efficient cellular energy supply confers a youthful appearance and a natural glow to the skin (Fig 1).

Mitochondria activation is the key to energise the skin for a 'Glow of Youth'

Even under normal conditions, skin has a high energy requirement to support its metabolic needs for tissue maintenance. But sometimes our life goes so fast that our skin cannot even follow. Insufficient supply of energy and over-production of ROS

induces accumulating oxidative damage. If the skin's countervailing mechanisms cannot keep pace, our skin not only becomes dull and lifeless but also develops visible signs of ageing.³

Mitochondria play key roles in cellular bioenergy and are known as biochemical powerhouses. Cosmetic strategies targeting the mitochondria will thus help to strengthen and energise natural skin functions to reveal a younger, healthier, more vibrant and vital radiance. Indeed, polyphenols from caper, mulberry and rose root have a great potential to combat the age-associated decline in energy production and to speed up the recharging of the skin's energy reservoir.⁷

The active ingredient combines extracts from caper, mulberry and rose root in a preservative-free and self-preserving solvent system (Fig 2):

Caper flower buds - Mitochondria activation power from the bud

The flower buds, better known as capers, have already been suggested by Arabian traditional medicine for the treatment of skin diseases, probably because of their high number of bioactive constituents, such as rutin, which accounts for about half of the total amount of phenolic compounds.^{8,9} Rutin counteracts ageing-related mitochondrial dysfunction: it increases the size and number of mitochondria¹⁰ and

boosts their activity as evidenced by increased oxygen combustion and energy production.¹¹ In addition, rutin counteracts physical fatigue. Extreme exercise is associated with a depletion of energy along with an increase of ROS. Rutin counteracts these key processes and thus has excellent anti-fatigue activity.¹²

Rutin levels are highest in small capers and decrease with their size. Rutin is degraded during industrial processing of capers, and fruits contain about six times less rutin than capers.¹³ For the active ingredient, we only use small and unprocessed caper flower buds. With this, we achieve a high level of bioactive ingredients for optimal mitochondrial activation (Fig 3A).

Mulberry leaves – Mitochondria protection and skin lightening

Morus species are valued for their leaves, which are nutritious, tasty and non-toxic,¹⁴ and constitute the main food for silkworms. In the 18th century, large areas in Europe had been planted with mulberry trees in order to establish the silk production. The invention of Nylon terminated silk production and the mulberry gardens fell into oblivion. Mulberry trees, however, are still found in historical gardens today.

Nowadays, leaves from black, white and red mulberry are being used in Japan and Korea for tea, while they are used as traditional folk medicine in China and India. The value of mulberry leaves is attributed to phenolic compounds such as chlorogenic acid, gallic acid and, again, rutin.^{14,15}

Chlorogenic acid provides mitochondrial protection. It prevents stress-induced mitochondrial injury by reducing ROS production, activating the antioxidant enzyme SOD, and suppressing mitochondrial membrane depolarisation.^{16,17} The action of chlorogenic acid is further supported by gallic acid, which has a resveratrol-like positive effect on mitochondrial function.¹⁷

In addition, mulberry leaves act as a

Figure 2: Power unit for your skin: a three-component natural ingredient based on caper flower buds, mulberry leaves and rose roots aiming to increase mitochondrial energy production while limiting collateral emissions. This provides an extra boost of energy for an improved skin glow and

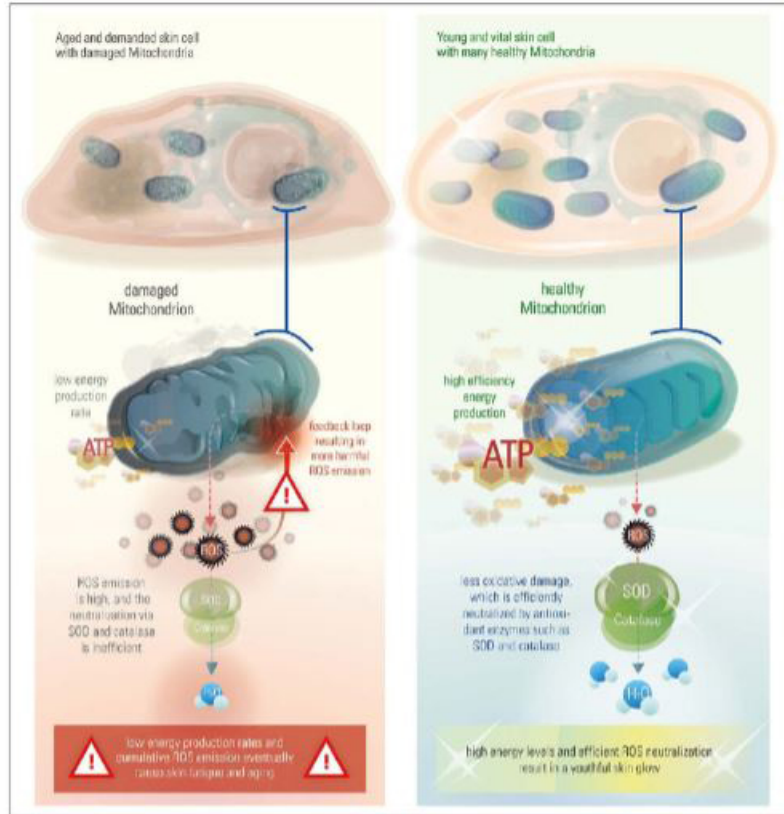


Figure 1: Mitochondria and ROS emission play a key role in skin ageing. Mitochondria are the powerhouse of cells, which produce energy in form of ATP. However, the process of mitochondrial energy production is not completely error-free and leads to the generation of oxidative damage (ROS emission). Mitochondria of aged and demanded cells produce less energy but have more collateral ROS emission. Moreover, ROS are less efficiently neutralised. The toxic ROS attack the mitochondria itself; this establishes a harmful feedback loop of cumulated oxidative stress, and eventually causes skin fatigue and ageing. In contrast, vital cells that contain many young and healthy mitochondria produce high levels of energy and low levels of ROS, while neutralising oxidative stress efficiently. This efficient energy production results in an invigorated skin with a youthful glow.

promising lightening agent against skin hyperpigmentation.^{18,19} Hence, they not only help to energise the skin via mitochondria protection, but also help to improve the skin's overall complexion and glow.

Rose roots - a mood-enhancing adaptogen with ergogenic function

Also known as arctic or golden root, its location and the process of extraction was a well-guarded secret. As late as in 1961, a Russian botanist located and identified golden root as *Rhodiola rosea*. Ever since, rose root received attention from the scientific community for its potential therapeutic capacity as an adaptogen and ergogen with putative anti-stress, mood-enhancing and performance-improving properties.^{19,20}

An adaptogen is a substance that increases our resistance to a variety of adverse physical, chemical or biological stressors in a non-specific way. An ergogen, like ginseng or caffeine, is a substance that enhances working capacity or exercise performance. Indeed, rose root counteracts exercise-induced fatigue and stimulates the recovery.²¹ Mitochondrial activation and free radical mitigation are the most plausible mechanisms by which rose root improves physical performance and stress tolerance. This is feasible since metabolites such as

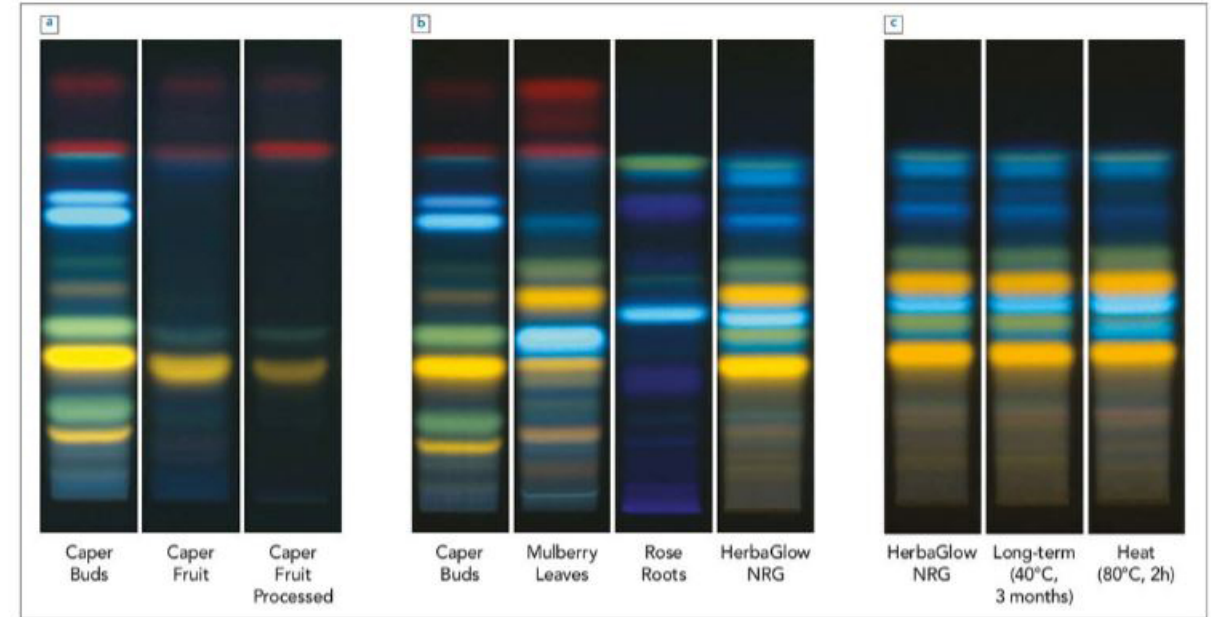


Figure 3: A synergistic and stable blend of energising metabolites. (A) HPTLC fingerprint of different caper-based extracts. (B) Illustrative, exhaustive extraction with methanol of caper buds, mulberry leaves and rose roots. The active ingredient concentrates these molecules in a natural solvent system. (C) Fingerprints of the active ingredient upon different stress conditions.

salidroside are well reported to stimulate mitochondrial function and to protect them against stress-induced dysfunction by activating ROS removing enzymes.²²

Phytochemical analysis

Objective

To identify the main metabolites and to evaluate their stability.

Technique

HPTLC (high performance thin layer chromatography) was used to assess the overall flavonoid/ phenolic compound composition.

Results

The comparison of different caper extracts demonstrated that the active ingredient is

based on optimal starting material, i.e. high-quality caper flower buds. Of note, unprocessed caper buds contain the highest amount and the most versatile profile of valuable metabolites (Fig 3A).

Each plant contributes particular lead compounds in order to form an efficient cocktail of mitochondria-activating molecules. The presence of substantial

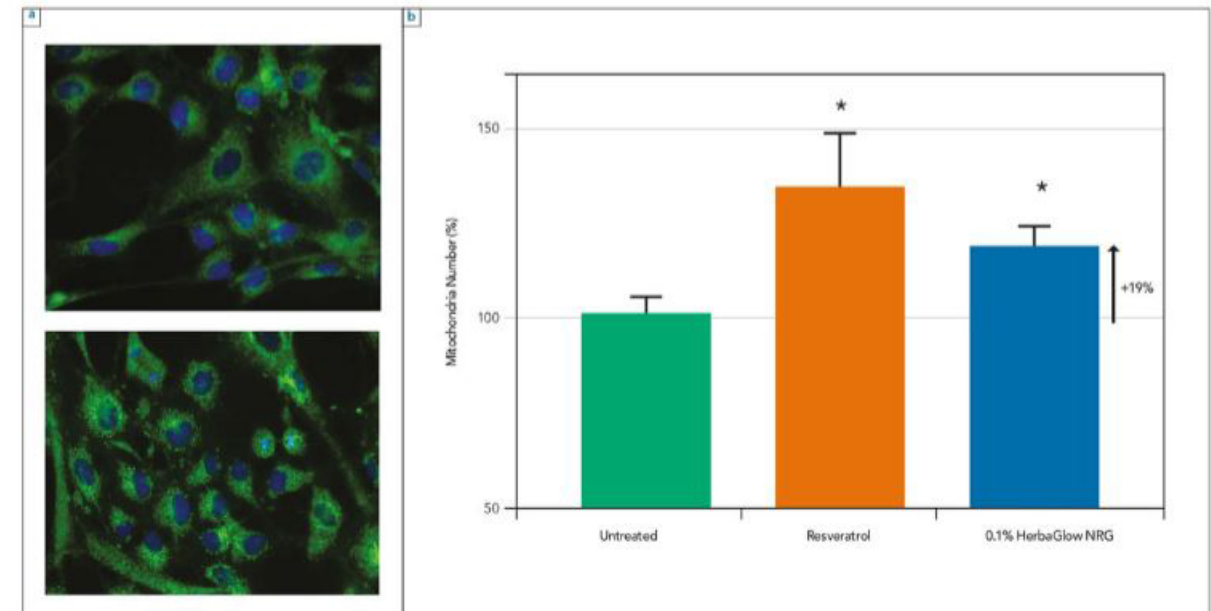


Figure 4: Boosting the numbers of mitochondria per cell. (A) Typical images of skin cells with low and high mitochondria numbers (top and bottom, respectively). The green fluorescence represents mitochondria; the nuclei are depicted in blue. (B) Human skin cells were incubated with 20 µg/ml Resveratrol (positive control²³) or active ingredient. N = 12 from two independent experiments; Mean ± SEM; Student's unpaired t-test versus untreated control; * = p < 0.05.

amounts of rutin, chlorogenic acid and salidroside was confirmed by dereplication analysis (Fig 3B and not shown).

HPTLC also demonstrated adequate heat and storage stability. Heating to 80°C for 2 h or storage at 40°C for 3 months had no effect on the overall flavonoid pattern (Fig 3C).

Increasing the number of mitochondria and improving energy supply

Objective
To show that the generation of mitochondria is stimulated.

Technique
Mitochondria Assay: human fibroblasts were incubated with test substances or not (untreated) for 72 hours, followed by staining with a mitochondria specific dye. The intracellular fluorescence was measured in multiple overlapping areas and normalised to protein content, which is a marker for the number of cells present in each culture. Increased fluorescence points to elevated numbers of mitochondria per cell.

Results
The active ingredient stimulates mitochondrial biogenesis: the number of mitochondria per cell increased by a significant ~20% (Fig 4B). Every cell of the skin is thus equipped with an extra portion of powerhouses. This, in turn, will increase the energy level of the skin.
Indeed, 0.01% of the active ingredient significantly increased the ATP level per cell by a significant ~20% (data not shown).

Improving the efficiency of energy production

Objective
To show that mitochondrial ROS emission is reduced. Harmful oxygen species are formed during energy generation in mitochondria. Increased mitochondrial numbers with increased energy production could consequently exhibit more oxidative stress.

Technique
DCF assay: ROS release was investigated by incubating human fibroblasts with test substances or not (untreated) for 72 h, followed by the addition of a non-fluorescing preliminary state of a dye that diffuses into the cell and is oxidised through ROS into a fluorescing product. A lower fluorescence signal thus indicates reduced mitochondrial ROS.

Results
The active ingredient provided additional energy while minimising side effects: skin cells with an increased number of mitochondria and higher energy production (see above) did not suffer from exaggerated

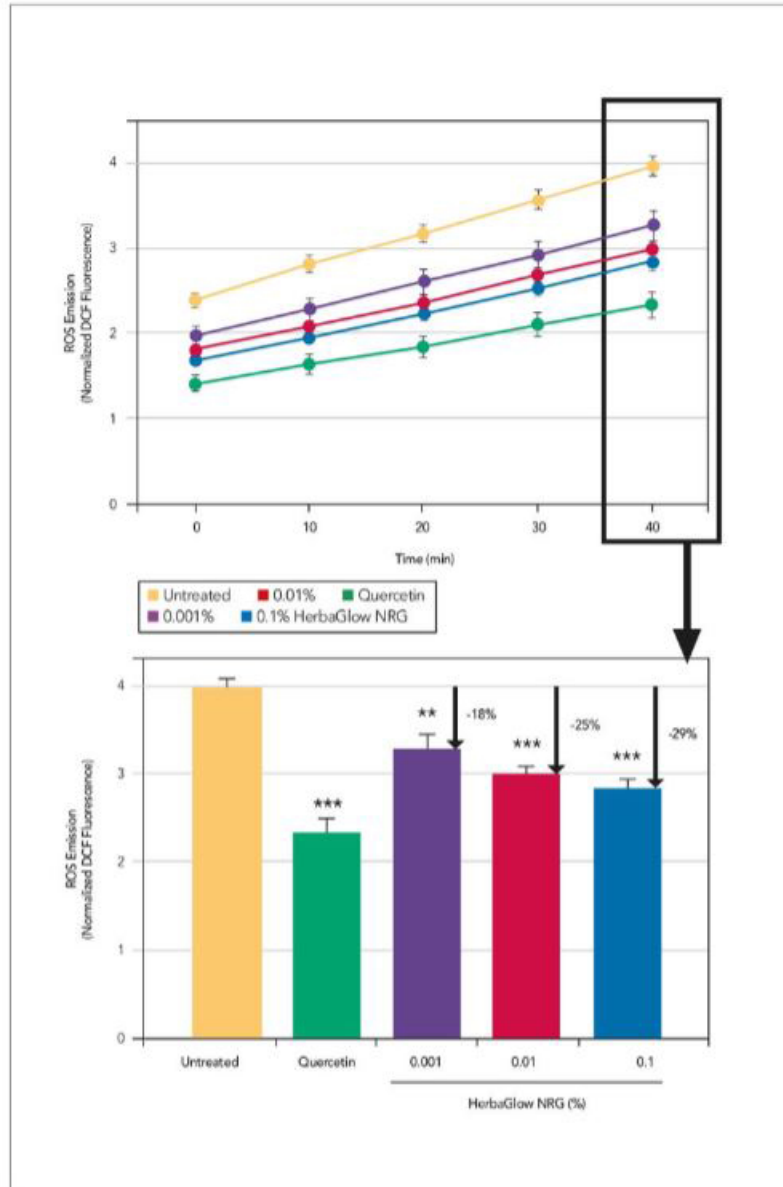


Figure 5: Reducing collateral ROS emissions. Skin cells were left untreated or were incubated with active ingredient or 3 ug/ml Quercetin (positive control²⁹). ROS release was determined over time (top) as well as at the end of the assay (bottom). N = 12 from two independent experiments; Mean + SEM; Student's unpaired t-test versus untreated control; * = p < 0.05; ** = p < 0.01; *** = p < 0.001.

ROS formation. The active ingredient even reduced free radical emission significantly and in a dose-dependent manner by up to ~30% indicating that energy production runs more efficiently (Fig 5). A more effective generation of energy with lower ROS emission is expected to result in a more youthful skin with recaptured glow and radiance.

Improving skin glow and radiance in vivo

Objective
To show that the active ingredient revives

the skin and offers glow and vitality. Skin with more energy is thought to be more radiant, which is a mirror of youth, health and happiness. Fabulous luminosity, homogenous complexion without imperfections, and smooth texture are all hallmarks of skin radiance.^{25,26}

There is no universal definition of skin radiance, but it appears to be the combination of:

1. Level of skin uniformity, including texture (smooth skin), colour and imperfections (no spots). Here, we show skin smoothness as evaluated by Primos.

2. Level of skin glow, including bright and youthful appearance. To this end, brightness, shininess and gloss were evaluated on the cheek using a glossymeter.
3. Vibrant skin colour, meaning skin with a nice hue that is neither overtly red nor yellow. Colour was evaluated by measuring L*, a* and b* on the cheek using a chromameter.
4. Physical and psychological wellbeing, which are subjective parameters. Wellbeing was self-assessed at the beginning and the end of the study using a 10-degree questionnaire that queries positive wellbeing, negative states of mood, and energy/ fatigue items.²⁷

Technique
Double-blind, placebo-controlled, randomised *in vivo* study with 2 x 20 Caucasian female volunteers, 30 – 65 years old. One group applied a cream formulation with 1% active ingredient, the other group applied the same formulation without active ingredient (placebo). Facial application, twice daily for four weeks. The following endpoints were assessed:

Results
The application of the active ingredient improved the facial skin radiance measurably, visibly and noticeably after 4 weeks:

The skin on the forehead and the cheek was significantly smoother (Fig 6) and became markedly more homogenous (not shown). The surface topography has a tremendous impact on the visual appearance. Firstly, even skin is thought to have better glow than uneven skin. Smooth skin is luminous and bright because it reflects light directly, whereas rough skin is dull because it diffuses light in many directions. Secondly, less wrinkles and particularly worry lines on the forehead will make the consumer look more happy and appealing – this, in turn, will have a positive impact on the subject's wellbeing.

Even skin reflected light more directly, which is a hallmark for radiant skin. As a consequence, the skin developed a healthy glow with a luminous and youthful touch (Fig 7 and not shown).

The overall skin colour improved substantially, as it became lighter but more intense – and with less pale yellow but more attractive pink (Fig 8).

These skin radiance benefits were substantiated by self-assessment via a questionnaire. Of note, improved radiance had a big impact on the overall wellbeing and the quality of life: volunteers experienced better mental comfort, more energy and less fatigue, and pessimistic vibes such as bad mood or worries had been allayed (Fig 9).

Improvement of Forehead Smoothness

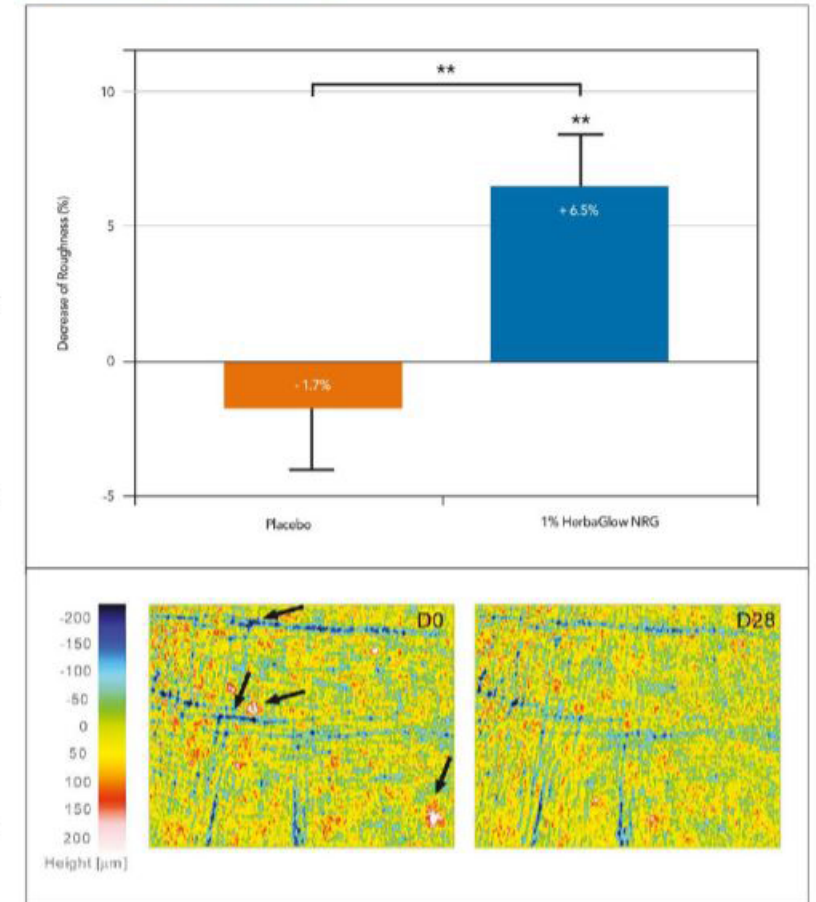


Figure 6: Providing smooth and uniform skin. Top: Relative decrease of skin roughness on the forehead after 4 weeks compared to untreated skin (before treatment). Bottom: Corresponding, representative pseudocolour images before and after treatment with the active ingredient. N = 20; Mean + SEM; Student's t-test versus baseline and between treatments; * = p < 0.05; ** = p < 0.01.

Improvement of Glow

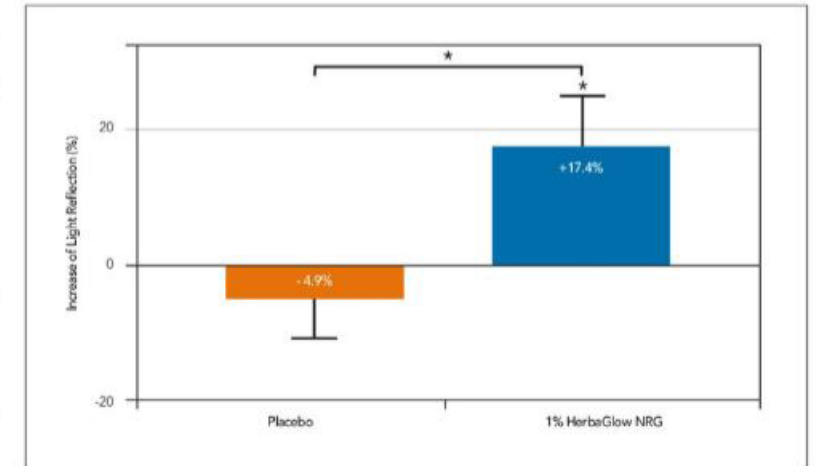


Figure 7: Providing brightness and a glow of youth. Relative increase of direct light reflection compared to untreated skin (before treatment). It is this mirror-like light reflection that gives the skin a natural, luminous gloss without appearing oily. N = 20; Mean + SEM; Student's t-test versus baseline and between treatments; * = p < 0.05; ** = p < 0.01.

Conclusion

Every organism requires sufficient energy levels to stay active and well protected. In particular, human skin cells, as being directly exposed to a multitude of environmental challenges, are highly dependent on large amounts of energy. A loss of skin energy is closely related to alterations in the skin structure – one main mechanism leading to premature signs of ageing.

Mitochondria are increasingly recognised as an important target for skin care, especially in the anti-ageing sector. Support of the mitochondrial biogenesis and function increases the energy level and helps to use cellular energy more efficiently. It is thus reasonable to expect that skin care products boosting mitochondrial functions will provide skin benefits such as oxidative defence, improved skin barrier, protection from environmental assaults, and ultimately rejuvenation of skin appearance.

We here demonstrated that mitochondrial activation by a natural active ingredient based on caper flower buds, mulberry leaves, and rose roots not only fights low energy levels, but restores the skin's natural glow visibly. The skin looks healthier, more radiant and younger, and the consumer feels fresher and happier. In essence, the active ingredient recharges and revives the skin and allows us to face the stresses and strains of everyday life with calmness – and attractive skin. PC

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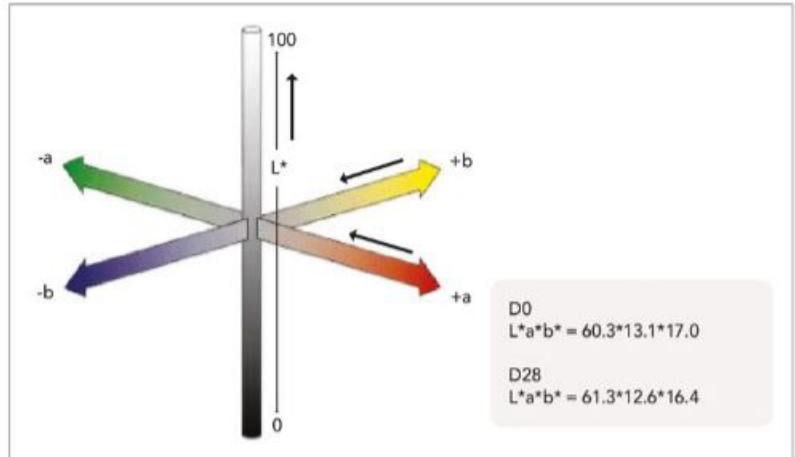


Figure 8: Allowing the skin to display its perfect colour. The application of the active ingredient for 4 weeks on the cheek led to a significant increase of the L*-value and to a significant decreases of a* and b* values (exemplified by arrows). L* provides data on the skin's grey level (the lighter the skin the higher the value: 0 = black; 100 = white). a* describes green-red and b* describes blue-yellow distribution (the higher the value the redder or yellower the skin). The application of placebo, in contrast, had no significant impact (not shown). N = 20; Mean + SEM; Student's t-test versus baseline and between treatments; * = p < 0.05; *** = p < 0.001.

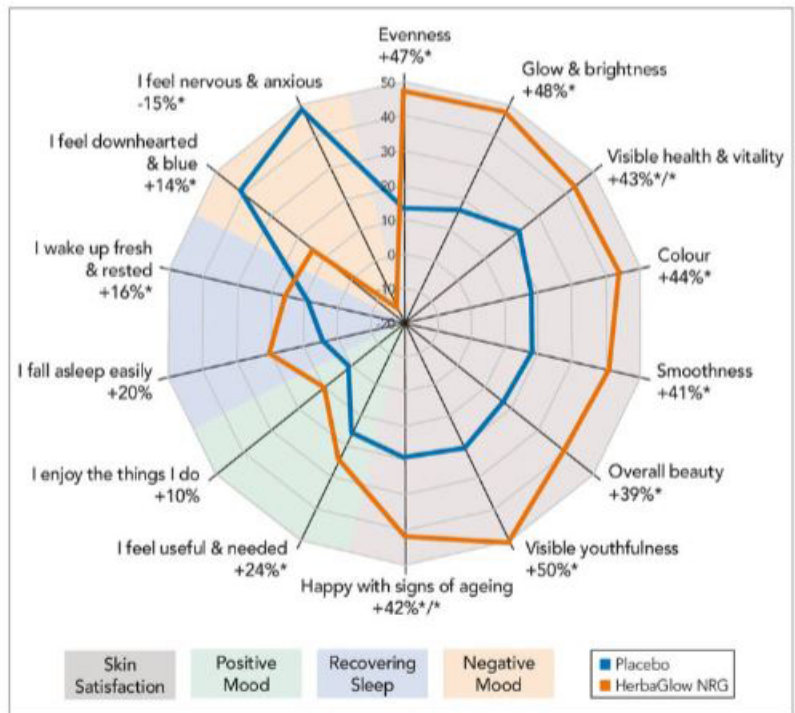


Figure 9: Making a crucial difference to skin and life satisfaction. Percentage of improvement for subjective scoring compared to before treatment. Upon the application of a cream with active ingredient for 4 weeks, the volunteers were significantly more satisfied with their skin appearance (grey), experienced better mental comfort (green), more energy and less fatigue (blue), and less pessimistic vibes (red). N = 20; Mean; Student's t-test versus baseline. * = p < 0.05.

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