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Natural Skin Barrier Supplement to Resist Artificial Radiation

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abstract

Artificial radiation is all around us. This refers not only to high energy visible light emitted by all kinds of screens but also WiFi radiation of smart devices. As we cannot escape from it and the consequences for our skin are still poorly investigated, protective measures can be taken in advance. Here we describe the use of an alga extract enriched in carotenoids capable of keeping the radiation threats away from our skin. The extract reduces WiFi and blue light-induced ROS generation and prevents overly carotenoid loss of the skin barrier leading to significant reduction in ageing parameters.

Introduction

It's true that our skin is strong and resilient. And we also know that balanced nutrition that includes a lot of antioxidants keeps the skin healthy. But what do we need to protect ourselves against? Two processes can generate reactive oxygen species (ROS) in the skin which can be eliminated by powerful antioxidants: The intrinsic process called respiration which happens in the mitochondria and extrinsic radiation, which lifts molecules in a higher energetic state. In both processes, the generation of singlet oxygen can be the starting point to produce a set of deleterious ROS which cause cell damage. The cell has found its way to cope with an increased ROS level. Via the nuclear receptor PPAR γ , the enzyme catalase is induced to eliminate hydrogen peroxide, a product of the sodium dismutase (SOD), eliminating the singlet oxygen. It is important to control the level of hydrogen peroxide as this can produce two dangerous hydroxyl radicals when in contact to metal ions. Hydroxyl radicals can do serious harm to DNA, proteins and lipids.

While the living parts of the skin, the cells, have their natural protection system, the corresponding enzymes are not present in the skin barrier, which is similar to the cell's architecture in that it is composed of lipid bilayers and hydrophilic parts in form of the natural moisturisation factor. This does not mean that there is no protection. Here, hydrophilic and lipophilic antioxidants are incorporated to protect the valuable molecules from damage. One of the most important class of antioxidants are the carotenoids. While our body is not able to synthesise them, they are absorbed from the food and deposited on the skin surface via sweat and sebaceous glands [1, 2]. From there, they can penetrate the lipid membranes of the skin barrier and fulfil their function. Carotenoids are originally invented by plants to protect their photosystems from high energetic blue light. As such, these molecules build the first line of defence against HEV light on the skin as well. This is well documented by the fact that carotenes bleach in sunlight and that the carotenoid content in the skin drops in summer.

But what about all the other forms of radiation to which your skin is exposed, nowadays? You might say that we already protect ourselves with a UV sunscreen as part of the daily skin care to be protected against UV radiation. However, all kinds of electronic devices, including smartphones, computer monitors, television and so on are constantly emitting harmful radiation. This can be HEV light or the very prominent WiFi or bluetooth radiation and both can severely damage the skin barrier by helping generate reactive oxygen species (ROS). Surprisingly, blue light in itself does not seem to represent a major risk to cells. However, there are numerous molecules in our tissues that act as photosensitisers. These include riboflavin (vitamin B2) and FADH but also the age pigment lipofuscin [3] or advanced glycation end products (AGEs) [4]. As a result, skin ageing becomes a self-reinforcing process when ROS-inducing

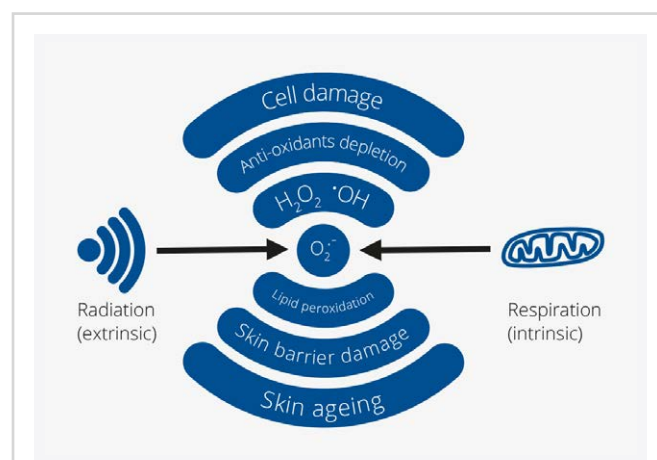


Fig. 1 External radiation and intracellular generation of ROS leads to a cascade of events. Starting with superoxide, highly reactive radicals emerge which destroy the antioxidant shield of the skin barrier and cells leading to substantial damage. The result is premature skin ageing.

radiation is also present. Here, modern technology may very well accelerate skin ageing. Smart devices use WiFi radiation for permanent accessibility. We must take into account that this radiation has a frequency of 2.4 GHz, which exactly resembles the radiation produced by a microwave oven to boil water. As such, at least tissue warming will be the result when we are exposed closely to WiFi emitters. Our own experiments revealed that this radiation creates ROS in keratinocytes even when the tissue warming factor was eliminated.

The carotenoid Faraday shield on our skin may thus quickly be exhausted and we should take care to reinforce it with the most obvious material: natural carotenoids in form of β -carotene and lutein enriched in RADICARE®-GOLD (INCI: Crambe Abyssinica Seed Oil, Beta-Carotene, Xanthophylls, Tocopherol, Helianthus Annuus (Sunflower) Seed Oil, Rosmarinus Officinalis (Rosemary) Leaf Extract), a supercritical CO₂ extract from the fresh water alga *Tetrademus obliquus*. In algae exposed to light stress, β -carotene is rapidly upregulated [5] and it has been shown that β -carotene is the first defence line of algae against the effects of blue light [6]. Hence, this green alga is the perfect choice when it comes to reproducing the radiation protection mechanisms employed by plants and using these for the purposes of defending the human skin.

Materials and Methods

Activation of PPAR

GAL4-PPAR activation assay with luciferase reporter gene activation in the standard reporter cell line 3T3-L1. The cells were transfected with a luciferase gene containing plasmid with a PPAR binding site. Following binding of a PPAR in the presence of active, the quantity of produced luciferase and thus the activity of the PPAR receptors was determined [7].

Blue Light Protection

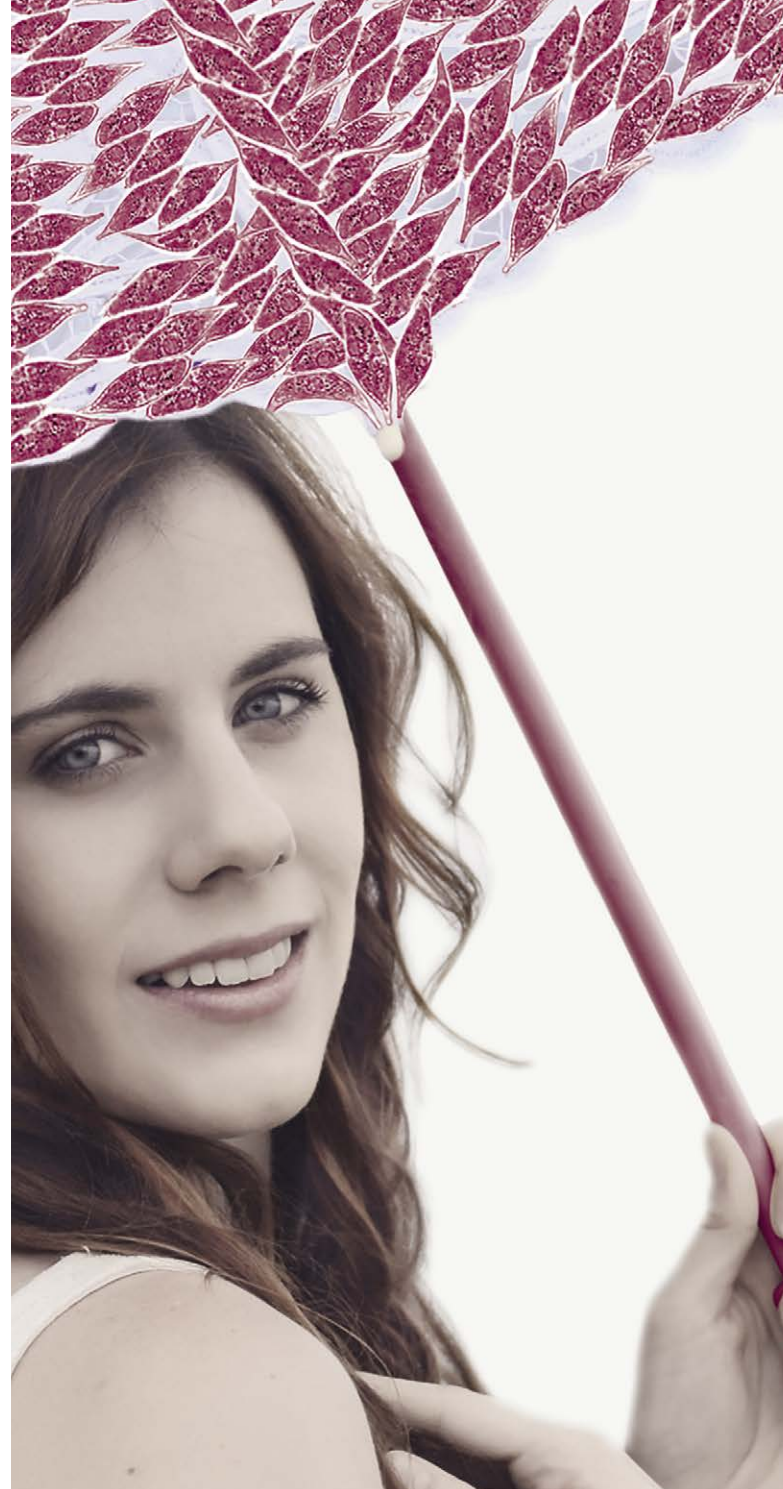
HaCaT cells were supplemented with 0.005% *T. obliquus* carotenoids (i.e. equivalent to 0.5% active) and irradiated with blue light at 450–470 nm for 120 min at 13 J/cm² (1.8 mW/cm²). Intracellular ROS production was measured with the DFFH-DA assay.

WiFi Irradiation

HaCaT cells were irradiated with WiFi radiation for 5 hours directly after application of the *T. obliquus* carotenoids. Subsequently, levels of intracellular reactive oxygen species (ROS) were determined using the Cellular Reactive Oxygen Species Detection Assay Kit (DCFDA).

Epidermal Strengthening

Scratch test using confluent HaCaT cells. A scratch was made using a 96-pin IncuCyte® WoundMaker resulting in a free space in the culture lawn. Active ingredient or medium containing FBS were added and images were recorded every 3



RADICARE®-GOLD

Your Personal Faraday Shield

NEW

- Blocks ROS caused by Wifi radiation
- Refills carotenoid depot in the skin
- Increases barrier strength

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hours. The degree of confluence was calculated using the IncuCyte® HD imaging system.

In vivo Studies

The *in vivo* studies were conducted in accordance with the World Medical Association’s Declaration of Helsinki. All study participants signed a written informed consent at the beginning of the study.

Carotenoid Loss

Carotenoid content of the forearm skin was measured by multiple spatially resolved reflection spectroscopy (MSRRS) using a Biozoom scanner [8].

Assessment of Standard Skin Parameters

Skin hydration was determined on the inner side of the forearms using a corneometer MPA 5 CPU. Skin elasticity was in the same way using a cutometer MPA 580. Skin roughness was determined using a PRIMOS 5.7 high-res device.

Statistics

For the determination of statistical significance, Student’s t-tests (unpaired for *in vitro* studies and paired for *in vivo* studies) were performed. Black asterisks refer to comparison with initial condition.

Results

The main intrinsic ROS source is the respiratory chain. Atmospheric oxygen can react with an escaped electron to superoxide and is then converted to hydrogen peroxide by superoxide dismutase. PPAR γ is a key nuclear receptor which regulates genes in response to oxidative stress. Activation of PPAR γ increases cat-

alase, which eliminates hydrogen peroxide to prevent apoptotic cell death [9]. PPAR γ as well as PPAR α are responsible for keratinocyte differentiation, wound healing and epidermal barrier recovery/maturation [10, 11].

Tetrademus obliquus carotenoids caused concentration-dependent activation of both PPAR α and PPAR γ (Fig. 2). A 1.4- to 1.7-fold increase was observed in the presence of 0.1 % active ingredient while a 4.3- to 5.4-fold increase was observed at 1 % active ingredient.

The resulting reduction of oxidative stress (not shown) leads to the stimulation of keratinocytes. The active ingredient significantly increased the proliferation capacity of HaCaT cells to recover in a scratch test by 52 % ahead of that of the untreated control (Fig. 3). The result demonstrates that induction of PPAR receptors and reduction of oxidative stress is likely to have a rejuvenating effect on the epidermis.

There is currently very little data on the potential harmful effects of WiFi radiation on human tissue [12]. Our lifestyle requires the continuous carrying of electronic devices which emit different kinds of radiation, among which WiFi radiation at 2.4 GHz is the most abundant one. Although the energy is low – just 0.5 mW in normal mode and more than 50 mW when connecting to an access point – it has not yet been investigated whether and how continuous exposure could affect our skin [13].

In non-irradiated keratinocytes, 0.005 % *T. obliquus* carotenoids already reduced the ROS content significantly by 11 %. Exposure to a 0.5 mW WiFi field for 5 hours significantly increased the internal ROS content by 12.4 %. This

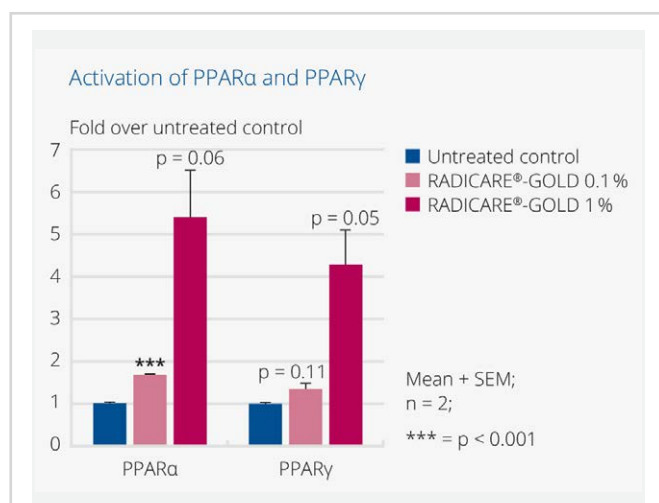


Fig. 2 Activation of PPAR α and PPAR γ . A concentration-dependent increase in activity was observed in presence of active ingredient.

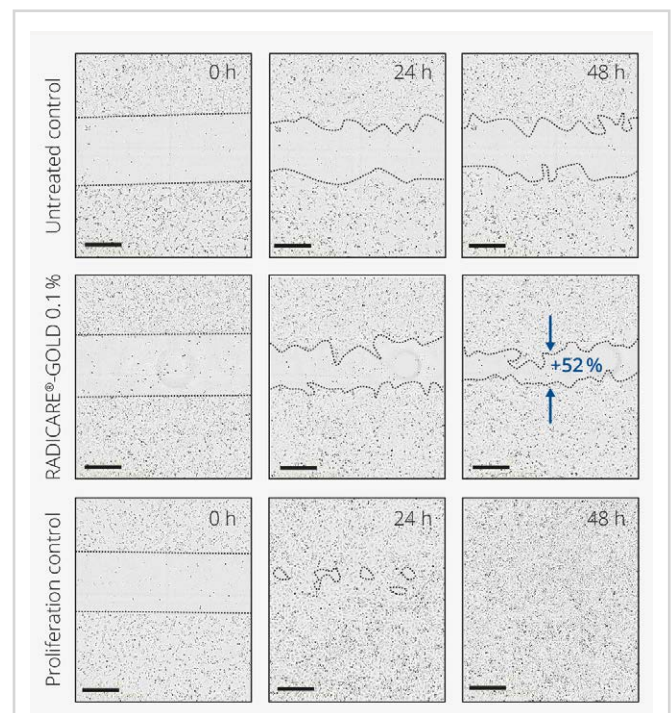


Fig. 3 The active ingredient promotes lateral keratinocyte proliferation. The active at 0.1 % showed a 52 % significantly faster recovery of the scratch as compared to control. The hyper-proliferation control also recovered very quickly, as was expected. The black bars represent 400 μ m.

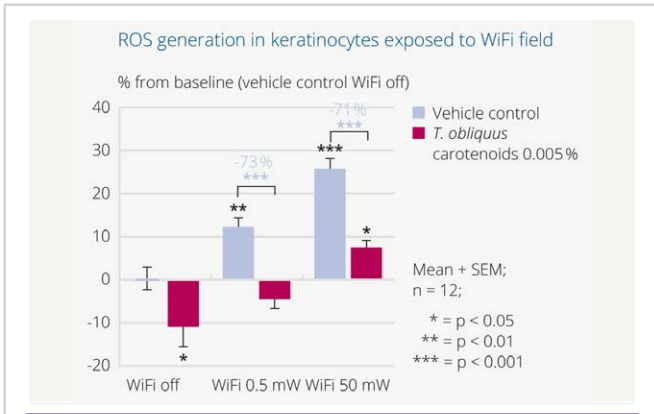


Fig. 4 *T. obliquus* carotenoids reduce ROS generated in a WiFi field. Exposure to a WiFi field dramatically increases intracellular ROS production which can be eliminated by the carotenoids.

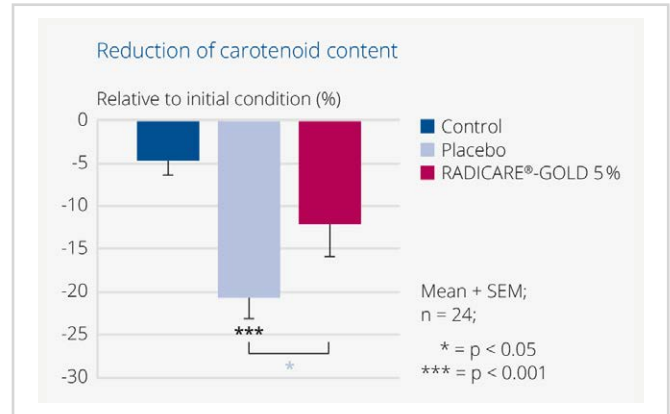


Fig. 6 The active ingredient supplements the skin barrier's anti-oxidative system. Application of the active protected the skin from significant carotenoid loss during summer.

was reduced by 73 % in the presence of 0.005 % *T. obliquus* carotenoids (Fig. 4). In the 50 mW mode, internal ROS levels increased dramatically by more than 25 %. The carotenoid supplement was able to reduce this additional ROS load by 71 % to only 7.5 %. We conclude from this that the active ingredient is capable of protecting the skin against electromagnetic radiation from mobile phones and other WiFi sources. It has been shown that carotenoids protect the skin against damage induced by sunlight [14]. Carotenoids are an important part of the molecular defence system of the skin barrier against ROS and free radicals. They are specialised in converting HEV light into harmless red light or vibration energy. A high carotenoid content in the skin positively correlates with the amount of collagen and elastin in the dermis [15], indicating a shielding effect that reduces radiation damage in the deeper layers of the skin. However, carotenoids – as well as other antioxidants – are depleted by solar radiation, especially in summer. Blue light is HEV light able to excite photosensitisers. These excited molecules transfer their energy to oxygen species and create ROS which cause cell and barrier damage. Consequently, irradiation of HaCaT cells with 13 J/cm² blue light at 450–470 nm increased the number of ROS-positive cells by 175 %. Supplementation with 0.005 % *T. obliquus* carotenoids resulted in the reduction of intracellular ROS by 42 % (Fig. 5).

The active not only protects the skin from the deleterious impacts of radiation but additionally helps to recharge the natural anti-oxidant shield of the skin. Compared to the baseline value in spring, the carotenoid content of the forearm skin treated with placebo dropped significantly by more than 20 %, indicating a depletion of the valuable antioxidants due to increasing sun radiation levels. In contrast, application of 5 % active ingredient led to a non-significant 12 % loss of carotenoids (Fig. 6). This represents 41.7 % lower depletion of carotenoids in comparison with placebo. Nutrition did not interfere with the measurements as can be read from the control value (thenar measurement). Sun exposure leads to functional deficits of the skin barrier leading subsequently to dehydration [16, 17]. The depletion of carotenoids due to sun exposure additionally results in a reduction of collagen and elastin in the dermis due to upregulation of MMP-12, the solar elastosis [15, 18]. This causes the typical signs of ageing, such as rough skin, less elasticity and the development of wrinkles. After both 14 and 28 days of treatment, there were significant increases by almost 50 % in skin hydration relative to initial condition and placebo. Positive effects were found in 100 % of the test subjects (Fig. 7). In addition, treatment with the active led to significant increases in skin elasticity. Although both placebo and verum

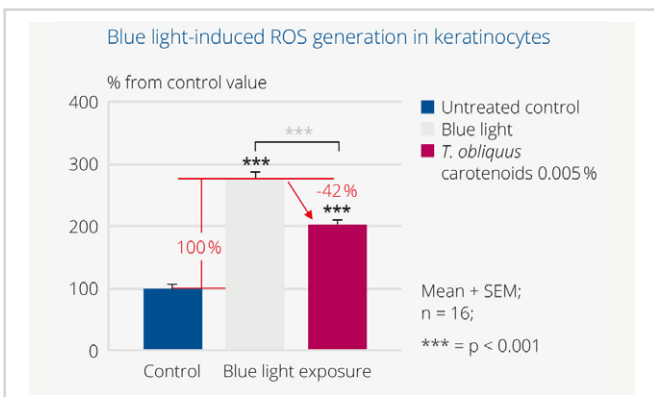


Fig. 5 *T. obliquus* carotenoids reduce blue light-induced ROS generation in keratinocytes. Supplementation with 0.005 % *T. obliquus* carotenoids resulted in a highly significant reduction of blue light-induced ROS.

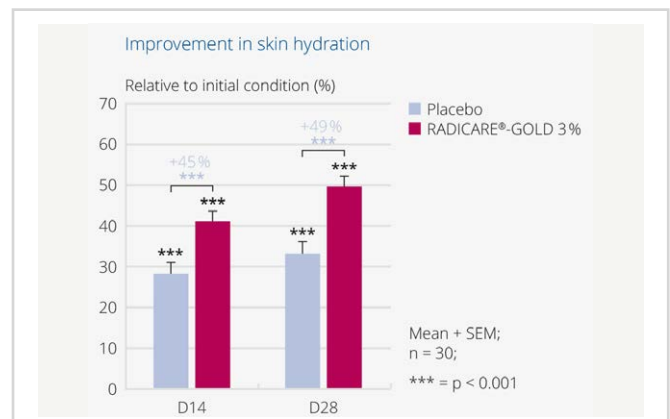


Fig. 7 Improvement of skin hydration. A significant improvement by almost 50 % compared to baseline and placebo was achieved after only 14 or 28 days.

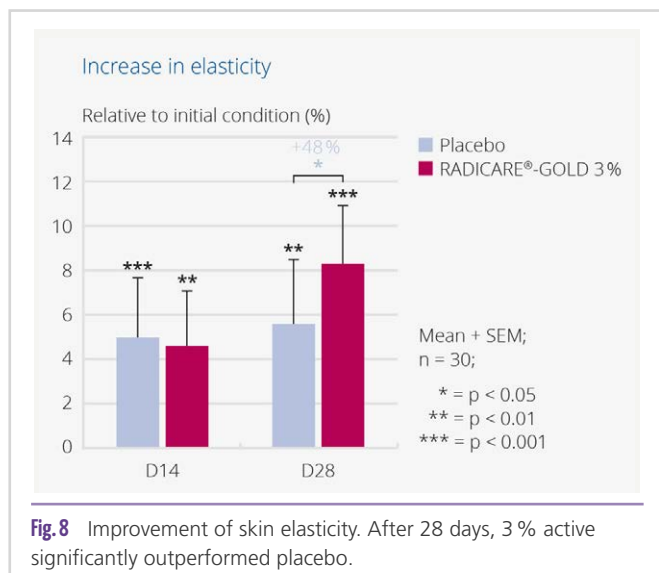


Fig. 8 Improvement of skin elasticity. After 28 days, 3 % active significantly outperformed placebo.

performed equally after 14 days, there was a significant increase in skin elasticity by 48 % in comparison with placebo after 28 days (**Fig. 8**). There was greater reduction of roughness by 58 % in comparison with placebo after only 14 days, and roughness continued to decrease after 28 days (**Fig. 9**).

Discussion

In a world in which sun radiation levels are apparently on the rise and there are obviously increasing levels of exposure to artificial radiation, RADICARE®-GOLD provides protection for the skin. It acts rather like a Faraday shield to block either deleterious radiation in the skin barrier while its consequences – elevated ROS levels inside the cells – are eliminated. The cells' intrinsic ROS defence system is augmented, leading to a

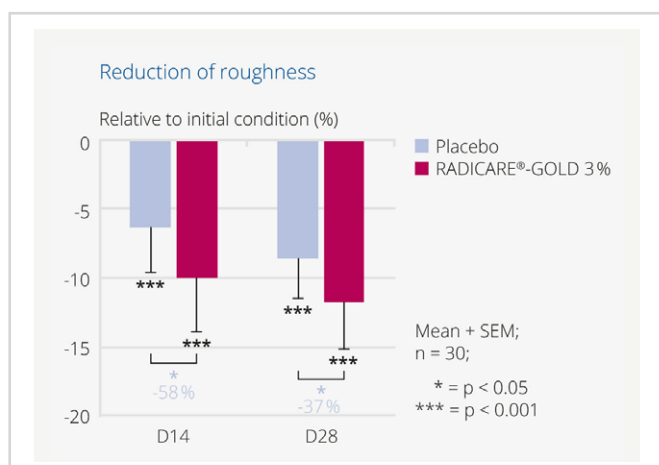


Fig. 9 Improvement of skin roughness. The roughness parameter Rz improved by almost 12 % after 28 days compared to initial condition. After only 14 days, the active was significantly more effective than placebo. Placebo.

reduction of oxidative stress. The depleted antioxidant depots in the skin are replenished, lipid peroxidation is reduced and the skin barrier is enhanced (published elsewhere). Because of this, dermal structures are protected, the skin stays elastic, becomes smoother and is rejuvenated.

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