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A personal Faraday shield for a radiant, high-tech world

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It has long been known that radiation is the main extrinsic cause of skin ageing as it attacks the skin barrier and increases the oxidative stress inside cells by inducing the formation of ROS.

The main source of radiation is the sun, which emits radiation of all wavelengths (Fig 1). If all of this radiation were able to get through to the Earth's surface, it is probable that higher forms of life would not have evolved on our planet. Luckily, the atmosphere absorbs most of the radiation and only a small fraction penetrates to the ground where it can harm our skin. Nonetheless, the residual UV radiation and the high energy fraction of visible light (HEV), in fact blue light, can be harmful to the skin. This is demonstrated by the fact that we become sunburnt if we expose ourselves for too long to the sunlight. The skin pigment melanin and of course the sun protection factors in cosmetic products are meant to protect us against UV radiation but there is no effective sunscreen for blue light on the market, yet.

Digital skin ageing in the modern world

HEV light is not only produced by the sun. Recent studies have shown that exposure to the sort of displays present in TVs and smart devices that also emit blue light can result in severe insomnia as the production of the sleep hormone melatonin is suppressed.¹ Furthermore, it has been clearly demonstrated that blue light is able to induce ROS formation in epithelial tissue,² as such it may advance premature skin ageing.

In addition to light in the visible and infrared spectrum, the sun also emits microwaves and radio waves. However, their energy is too low to have any damaging effect on our body as they are mostly blocked by the atmosphere. As a consequence, our skin has not naturally developed ways of dealing with this potential threat. The effects of the artificial overload of these forms of energy created by us humans on Earth - so-called

Abstract

Radiation outside the UV-range is one of the most unexplored threats for our skin. For sure, we protect ourselves against UV light but forget the high-energy visible light fraction. However, not only blue light increases the ROS load in keratinocytes leading to skin barrier damage and premature skin ageing. Our modern, highly connected world with permanent access to the internet and communication devices emits a tremendous amount of radiation. A large fraction is WiFi radiation in the range of microwaves. To protect our cell membranes and skin barrier, we need anti-oxidants active in the depth of the membranes to prevent deep lipid peroxidation, followed by functional impairment of these structures. Radicare®-Gold is the skin's personal Faraday shield made from natural carotenoids to reduce the ROS load provoked from any source.



Figure 1: We are surrounded by all kinds of radiation from natural and human-engineered sources. Some rays are definitely deleterious to our skin although for most radiation no clear evidence of their harmful potential exists. Visible light is only a minor fraction of the radiation that hits the skin's surface.

'electrosmog' - are currently being investigated. There is no doubt that the radiation levels of our everyday devices - mobiles, computers, televisions etc. - are low. Nonetheless, the massive increase in the number of smart devices in use, the

extension of the mobile network and the use of mobile phones close to our bodies mean that continuous and potentially harmful irradiation of body tissue can occur. Although there is as yet no definitive proof, evidence is beginning to emerge that the

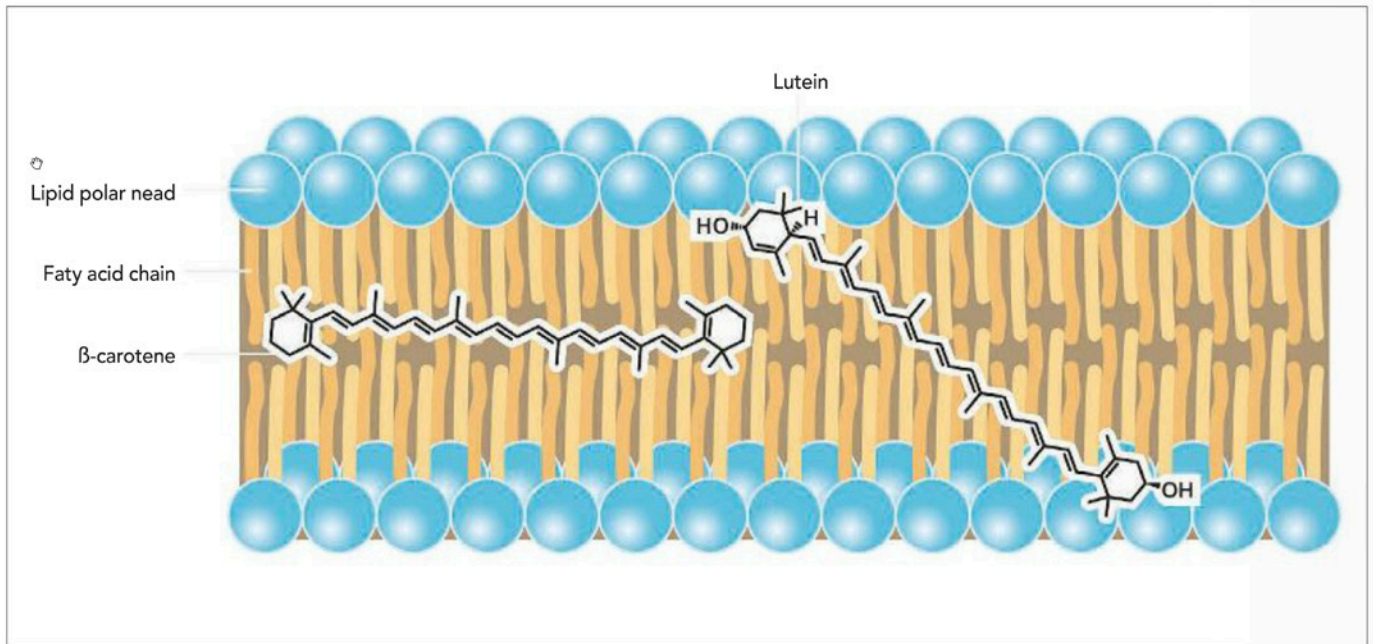


Figure 2: Carotenoids are incorporated in the lamellar lipid bilayer of the skin barrier and of cellular membranes. While carotenes without hydrophilic moieties are situated in the fatty acid chain layer, xanthophylls like lutein are integrated in the membrane with the polar head facing outwards.

frequencies used by mobile phones (GSM-900, GSM-1800, UMTS, LTE) may damage DNA, proteins and lipids³ and that the 2.4 GHz WiFi or Bluetooth radiation in the microwave range can significantly impact on gene expression.⁴ By exposing our skin to all these artificial forms of radiation, we could well be subject to digital skin ageing, a new threat to otherwise healthy skin. We need to take precautions to combat this novel kind of hazard.

The balance of intrinsic ROS-production and elimination

In every skin cell, atmospheric oxygen is converted to water in the respiratory chain of the mitochondria. This is the main intrinsic ROS source which is under tight control. It is generally the case that free radicals are unable to escape the respiratory chain.⁵ However, there can be 'leakage' of electrons and these react with atmospheric oxygen to create superoxide anions.^{6,7} This free radical is converted by the enzyme sodium oxide dismutase (SOD) to hydrogen peroxide. The level of hydrogen peroxide is an indicator of the intrinsic ROS stress level of cells. In presence of metal ions, hydrogen peroxide can dissociate into two very reactive hydroxyl radicals that can do serious harm to lipids and cellular components - consequently leading to skin barrier damage and premature skin ageing.

To eliminate this threat, the cells employ an efficient ROS defence system. This is controlled inter alia by PPAR γ , a nuclear receptor that increases levels of the enzyme catalase to eliminate hydrogen peroxide.⁸ Catalase converts hydrogen peroxide into non-dangerous end products like water and

UV/VIS spectrum of RADICARE®-GOLD

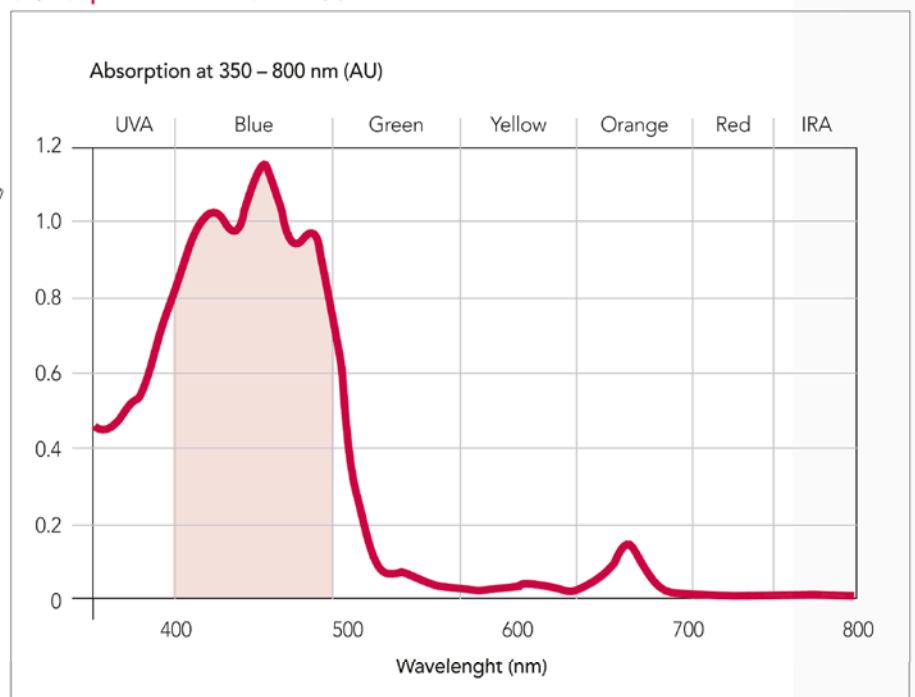


Figure 3: The UV/VIS spectrum of the active ingredient shows the typical results for carotene. β -carotene and lutein absorb light mainly at the blue light end of the spectrum with characteristic peaks at 400 - 490 nm. The peak at 680 nm was produced by a residual quantity of pheophytin a, a degradation product of chlorophyll.

atmospheric oxygen. Several other pathways prevent the formation of further radicals or eliminate them⁹ and natural antioxidants are part of this complex system. Of note, a low intracellular concentration of ROS acts on second messengers, gene regulators and mediators for cell activation. However, extrinsic ROS generating radiation can very well induce a breakdown of the system

The skin's own 'Faraday shield'

The epidermis of the skin is the visible part of our body that interacts with the environment. It is a very specialised kind of tissue that has developed to cope predominantly with external threats such as particles, chemicals, pollution in general, wind and weather and of course radiation, mainly that of the sun. These factors are the main causes of premature skin ageing¹⁰ and

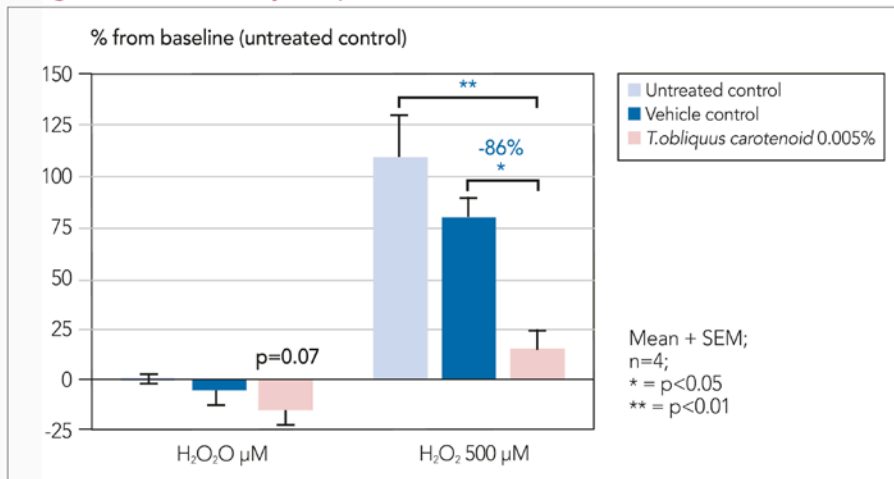
ROS generation in Keratinocytes exposed to H₂O₂

Figure 4: *T. obliquus* carotenoids can reduce the internal ROS load attributable to excess hydrogen peroxide. Cells were incubated with 0 μM or 500 μM hydrogen peroxide with and without 0.005% *T. obliquus* carotenoids. Internal ROS generation was brought back to an almost normal level in the presence of *T. obliquus* carotenoids while the vehicle DMSO did not have a significant influence on internal ROS concentration. Unpaired Student's t-test. The statistical values in black are the result of comparison with the untreated control while the blue value is the result of comparison with the vehicle control.

Protection against lipid peroxidation (cumene hydroperoxide stressed)

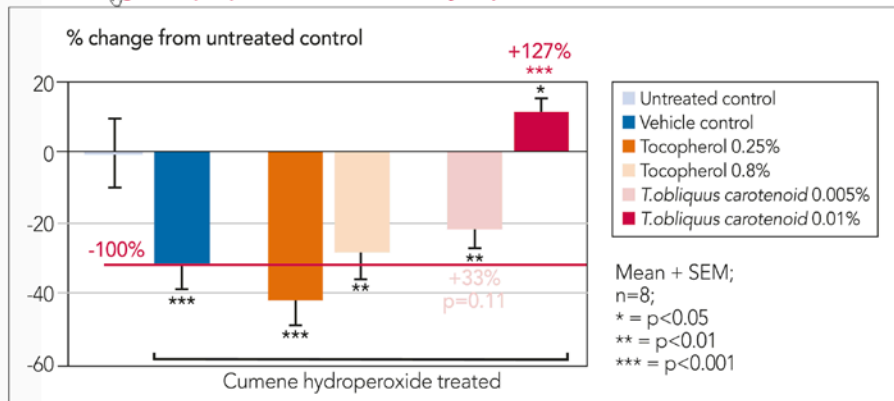


Figure 5: *T. obliquus* carotenoids protect against lipid peroxidation. Induction of lipid peroxidation with cumene hydroperoxide leads to an increase in lipid peroxidation or a decrease in the protective power of the cells against lipid peroxidation by 32%. Addition of tocopherol did not change this result. Addition of *T. obliquus* carotenoids fully protected the cells from the lipid peroxidation stress induced by cumene hydroperoxide. Unpaired Student's t-test. The statistical values in black are the result of comparison with the untreated control while the red value is the result of comparison with the vehicle control.

the skin has devised an effective countermeasure against these hazards. This is the very effective shield known as the skin barrier. The skin barrier is composed of corneocytes present in the lipids of the stratum corneum, the outermost layer of the skin. Additionally, there is a natural moisturisation factor (NMF) that also forms part of the skin barrier. While this facilitates skin hydration, the stratum corneum lipids provide a barrier against transepidermal water loss. If these lipids deteriorate, this will have a severe impact on the barrier function of the skin and lack of skin hydration and premature skin ageing will be the consequences. The major potential danger is photo-oxidative impairment of the skin barrier by UV and blue light; these are most intense in summer and at the equator, as the UV index shows. Our body

has developed a defence strategy against this threat: it employs antioxidants, mainly carotenoids, which are released on the surface of the skin.¹¹ This is to prevent radiation generated reactive oxygen species (ROS). These molecules readily undergo reactions and can cause lipid peroxidation. This will mean the skin barrier can no longer function properly while it will also become susceptible to further damage. Carotenoids are the most effective antioxidants when it comes to preventing the oxidation of substances such as squalene¹² and they are also highly efficient in blocking HEV light. As such, the skin is protected to a certain degree. But our body cannot produce carotenoids on its own. We have to ingest them by eating vegetables and these powerful antioxidants are then excreted in the sweat and from the

sebaceous glands and deposited where they are needed most.^{13,14} In this way, the skin produces its own 'Faraday shield' that protects it against radiation from natural sources.

Carotenoids: the perfect natural solution
We could say that the skin creates its own topical cosmetic formulation at the frontline of its battle with ROS in the form of the antioxidants it supplies to the skin barrier. What has worked for thousands of years may now have to be improved as today we encounter artificial radiation on a regular basis, we are living longer and we like to retain a youthful appearance as long as possible. As such, our natural antioxidant shield is constantly hard-pressed. In summer, increased sun exposure leads to an increased loss of carotenoids. Interestingly, not only blue light irradiation, but also fatigue, illness, smoking, and alcohol consumption have a negative impact on skin carotenoids.¹⁵ The new healthier nutrition trend can help us retain an appropriate level of carotenoids on our skin. However, we do not always have the opportunity to maintain the balance. Thus we need to supplement our skin to ensure we are fully protected. It is obvious that the appropriate cosmetic solution for this is to increase the amount of carotenoids in the stratum corneum by means of topical application of these substances in order to preserve the efficacy of the skin barrier. The result will be a more potent, more robust skin barrier with fewer detached corneocytes due to dry skin and scaling.

A perfect solution is to provide the skin with an additional reservoir of this important supplement in form of the natural carotenoids present in the active ingredient Radicare®-Gold (INCI: Crambe Abyssinica Seed Oil, Beta-Carotene, Xanthophylls, Tocopherol, Helianthus Annuus (Sunflower) Seed Oil, Rosmarinus Officinalis (Rosemary) Leaf Extract).

Radicare-Gold is an exclusive blend of natural β-carotene and xanthophyll (lutein) obtained from the fresh water alga *Tetrademus obliquus* (*T. obliquus* carotenoids).

Carotenoids are the most important natural antioxidants that neutralise molecules which undergo excitation on exposure to light or radiation because of the large conjugated π-electron system (the large number of nearby double bonds, see Fig 2). This can distribute excess energy resulting from excited electron states over the whole molecule and disintegrate it by simple vibration.¹⁶ Additionally, carotenoids can eliminate radicals by donating a hydrogen atom - just as other antioxidants do - but they can also donate an electron to a radical, which other antioxidants are not able to do.

Vehicle control

Cumene hydroperoxide 100µM

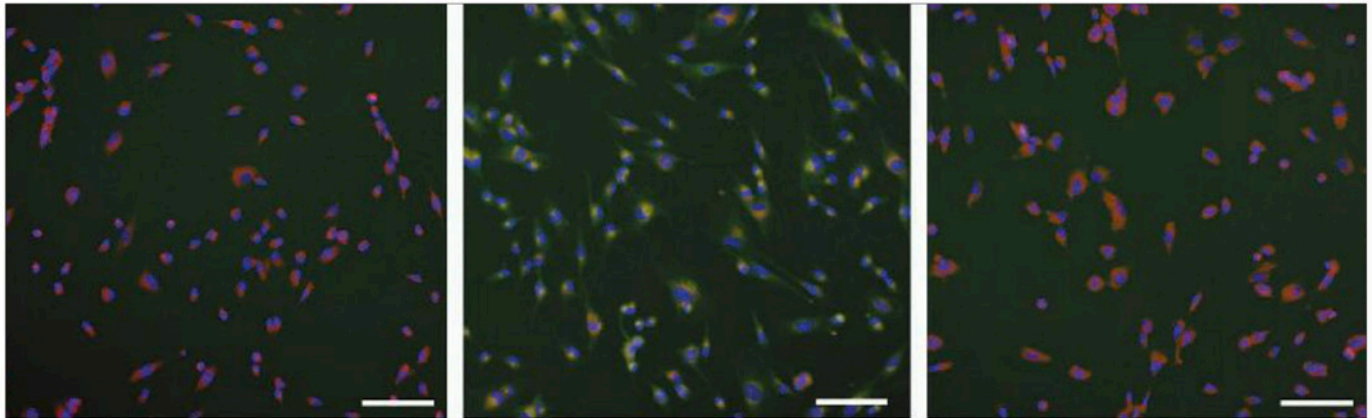
Cumene hydroperoxide 100µM + *T.obliquus* carotenoids 0.01%

Figure 6: *T.obliquus* carotenoids prevent lipid peroxidation. Epifluorescent images of primary human dermal fibroblasts. Cell nuclei are stained in blue. Vehicle treated cells contain only a minor fraction of lipid peroxides and are stained in red (left panel). Addition of 100 µM cumene hydroperoxide lead to a significant increase in lipid peroxidation, as can be seen by the colour change of the dye from red to green (middle panel). *T. obliquus* carotenoids provide full protection against cumene hydroperoxide induced lipid peroxidation (right panel). The white bar represents 100 µm.

There are two major types of carotenoids: those that are completely lipophilic are called carotenes and those with polar head groups (i.e. oxygen-containing carotenoids) are called xanthophylls. Lipophilic carotenoids such as β-carotene are incorporated in the lamellar skin barrier lipid system or are integrated between the lipid bilayers of biomembranes. In contrast, xanthophylls, including lutein, are substances located in the transmembrane region (Fig 2). This means carotenoid accumulation in the skin barrier provides a protective network for lipids in the lamellar lipid system. Carotenoids incorporated in cellular membranes inhibit lipid peroxidation and divert excess energy away from the

vulnerable unsaturated fatty acid tails of the membranes. β-carotene and lutein undergo synergistic interactions with each other and α-tocopherol, which can result in the regeneration of β-carotene.^{17,21} Tocopherol, in turn, is regenerated by the water soluble vitamin C. It is because of all this that it is important to maintain a fully functional network of antioxidants in our skin.

Methods

UV/VIS spectrophotometry: The cosmetic active was diluted 1:10 in 90% acetone and a UV/VIS spectrum over the range 350 - 800 nm was recorded.

Oxidative stress with hydrogen peroxide: Subconfluent, proliferating HaCaT cells were incubated with 500 µM

hydrogen peroxide for 20 minutes with or without 0.005% *T. obliquus* carotenoids in DMSO (corresponds to a 0.5% concentration of the active). Levels of intracellular reactive oxygen species (ROS) were determined using the Cellular Reactive Oxygen Species Detection Assay Kit (DCFDA).

Protection against lipid peroxidation: Primary human dermal fibroblasts were grown over night and incubated with *T. obliquus* carotenoids at 0.005 % or 0.01 % or corresponding DMSO vehicle controls, respectively. After 30 minutes, fluorescent staining reagent of the Image-iT® Lipid Peroxidation Kit and 100 µM cumene hydroperoxide were added. Lipid peroxidation was measured by fluorescent imaging and quantification.

Reduction of WiFi induced oxidative stress: For the irradiation experiment, a prototype WiFi emitter system was developed (Fig 7) that very realistically simulates the exposure of skin to the WiFi radiation of smart phone devices. Conventional WiFi emitters were placed beneath a 24-well cell culture dish at a distance of 10 mm. The radiation intensity was set to 0.5 mW or 50 mW, the typical everyday emission ranges of these devices. The experiment was performed under tight temperature control avoiding heating of the culture medium by irradiation and a Faraday cage shielded the surroundings from the WiFi radiation. Subconfluent, proliferating keratinocytes were placed on the WiFi emitters and irradiated 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).

Reduction of lipid peroxidation and



Figure 7: Cell culture WiFi emitter system within a Faraday cage.

desquamation *in vivo*: The *in vivo* study has been performed in accordance with the principles of good laboratory practice (GLP), good clinical practice (GCP), and in compliance with the quality assurance system requirements. The study was 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. 20 female subjects, all with healthy, Caucasian skin, aged 20 - 46 years (average 30.6 years) applied twice daily an emulsion containing 0% active (placebo) or 3% active on the hemiface in a double-blind, placebo-controlled, randomised study. For desquamation measurements, corneocytes were detached from the face with Corneofix® F20 desquamation collector foil at day 0 and day 28 and the mass of the collected corneocytes was measured. The collected corneocytes were then used for the Lipid Peroxidation (MDA) Assay Kit in order to measure levels of lipid peroxides.

Results

T. obliquus carotenoids absorb HEV light HEV or blue light induces ROS in the skin barrier and in skin cells. *T. obliquus* carotenoids absorb blue light in the range 400 - 480 nm, which represents the high

ROS generation in Keratinocytes exposed to WiFi field

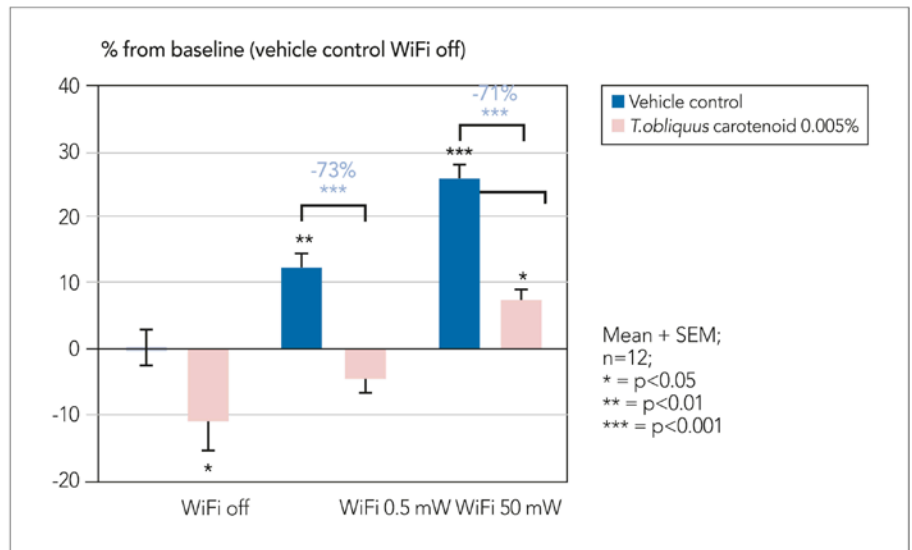


Figure 8: *T. obliquus* carotenoids reduce ROS generated in a WiFi field. Internal ROS production in keratinocytes irradiated with different intensities of WiFi radiation was measured. Internal ROS were quenched significantly by 10.9% without radiation (WiFi off). On exposure to a WiFi field, the generation of ROS increased with field intensity by up to +25% at 50 mW. *T. obliquus* carotenoids reduced this extra ROS load by more than 70%. Unpaired Student's t-test.

energy fraction of visible light, directly adjacent to UVA light (Fig 3). This means they can act in principle as a blue light filter. In final cosmetic formulations at a concentration of 1 - 5%, the level of

absorption will be reduced. Hence it can be assumed that in the final product, the ROS-eliminating capacity of the carotenoids will predominate over the beneficial effect attributable to screening



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of blue light irradiation. A significant ROS eliminating capacity was proven in an experiment irradiating keratinocytes with blue light (not shown).

T. obliquus carotenoids alleviate cellular oxidative stress

Exposure to blue light induces photosensitiser-mediated singlet oxygen generation which inactivates catalase.²² This leads to an accumulation of hydrogen peroxide, which promotes radical formation and subsequent cell damage.²³ Supplementation with 0.005% *T. obliquus* carotenoids suppressed hydrogen peroxide-induced radical formation in keratinocytes so that this returned almost back to a normal level (Fig 4). The vehicle DMSO did not have a significant influence on the results.

T. obliquus carotenoids protect cells against lipid peroxidation (*in vitro* study)

Carotenoids incorporate deeply into the lipid bilayer of cell membranes or other biomembranes, e.g mitochondrial membranes or lamellar lipid systems in the skin barrier (see Fig 2). As such, they should provide maximal capacity to protect the lipophilic moieties inside the membranes.

Induction of lipid peroxidation with cumene hydroperoxide leads to a significant collapse of the protective system of the cells (Fig 5, vehicle control). Tocopherol was not able to prevent lipid peroxidation while 0.005% *T. obliquus* carotenoids reduced the lipid peroxidation by 33% compared to vehicle control and 0.01% *T. obliquus* carotenoids reduced the lipid peroxidation significantly by 127% over vehicle control, even 11.5% superior over the non-treated condition (Fig 6).

Although tocopherol is an effective antioxidant in unstressed skin cells (not shown), it loses its anti-oxidative power in cases of deep lipid peroxidation. The reason might be that tocopherol cannot intercalate into phospholipid membranes as carotenoids can do. It is situated at the polar head groups of the phospholipids with its aliphatic tail aligning with the fatty acid tails. However, this tail does not have the capacity to quench radicals. As such, tocopherol can only protect against superficial damages of phospholipid membranes while carotenoids are incorporated deeply at the site of lipid peroxide generation.

T. obliquus carotenoids reduce WiFi-induced oxidative stress

There is currently very little data on the potential harmful effects of WiFi radiation on human tissue.³ Our lifestyle requires the continuous carrying of electronic devices which emit different kinds of radiation, among which 802.11g WiFi radiation at 2.4

Lipid peroxidation in corneocytes

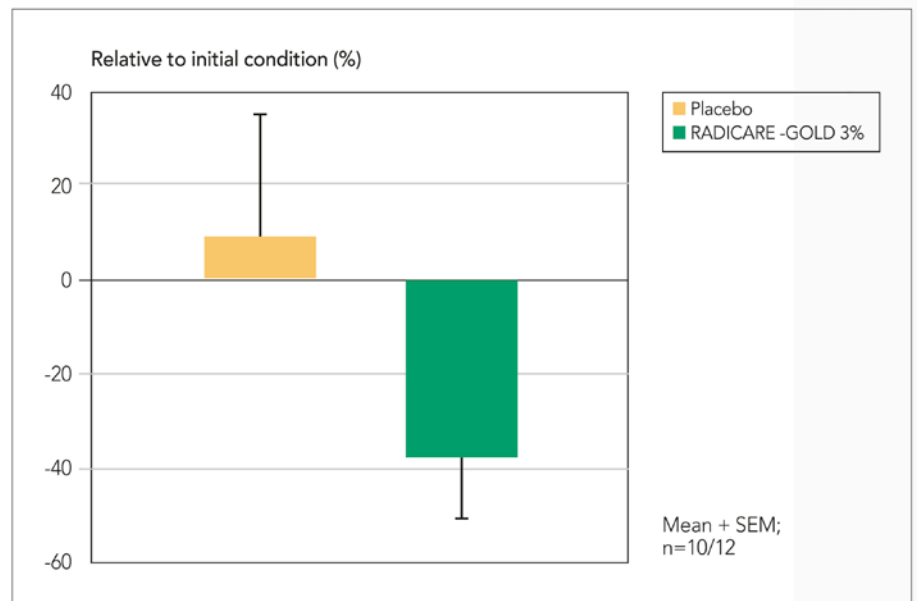


Figure 9: The active protects the skin against elevated radiation. Lipid peroxidation was slightly increased after application of placebo for 28 days in the summer, while treatment with 3% active reduced lipid peroxidation.

– 2.48 GHz might possibly be the most harmful. The frequency corresponds to that of the radiation emitted by microwave ovens and as such will cause tissue warming especially if the mobile device is carried close to the body. Although the energy is low (0.5 mW - 100 mW) it has not yet been investigated whether and how continuous exposure could affect our skin.²⁴ In non-irradiated keratinocytes, 0.005% *T. obliquus* carotenoids 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 was reduced by 73% in the presence of 0.005% *T. obliquus* carotenoids (Fig 8). The 0.5 mW power level corresponds to that of the normal mode of a smartphone with WiFi connection. Exposure to a 50 mW field corresponds to the search and connection process for an access point. In this mode, internal ROS levels increased 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 *T. obliquus* carotenoids are capable of protecting the skin against electromagnetic radiation from mobile phones and in the microwave band.

T. obliquus carotenoids reduce lipid peroxidation and strengthen the skin barrier

Elevated radiation during summer depletes the natural concentration of antioxidants in the skin.²⁵ This leads to lipid peroxidation causing a weakened skin barrier and dysfunction of skin cells. A topical supplementation of the skin with carotenoids can replenish a reduced

antioxidant pool and protect from lipid peroxidation. In this *in vivo* study, 20 female volunteers applied a placebo formulation and a verum formulation containing 3% of the active ingredient on their hemiface. Application of placebo in the summer led to a slight increase in levels of lipid peroxides in corneocytes after 28 days, whereas active-treated skin exhibited a reduction in lipid peroxidation by 37% (Fig 9). This result is consistent with the finding that a cosmetic formulation with the active is able to supplement the skin with carotenoids (not shown). The amount of corneocytes that could be detached with Corneofix® on the face was significantly reduced after application of the active to 31.3%. This is an almost 70% reduction of desquamation and increase in epidermal skin barrier function as compared to the initial condition (Fig 10). As a result, the strengthening of the skin barrier leads to a significantly more hydrated, elastic skin with reduced signs of premature skin ageing (not shown).

Conclusion

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 reduction of oxidative stress. Radiation such as UV and (artificial) blue light induce ROS formation in the lamellar system of the

Increase in barrier strength

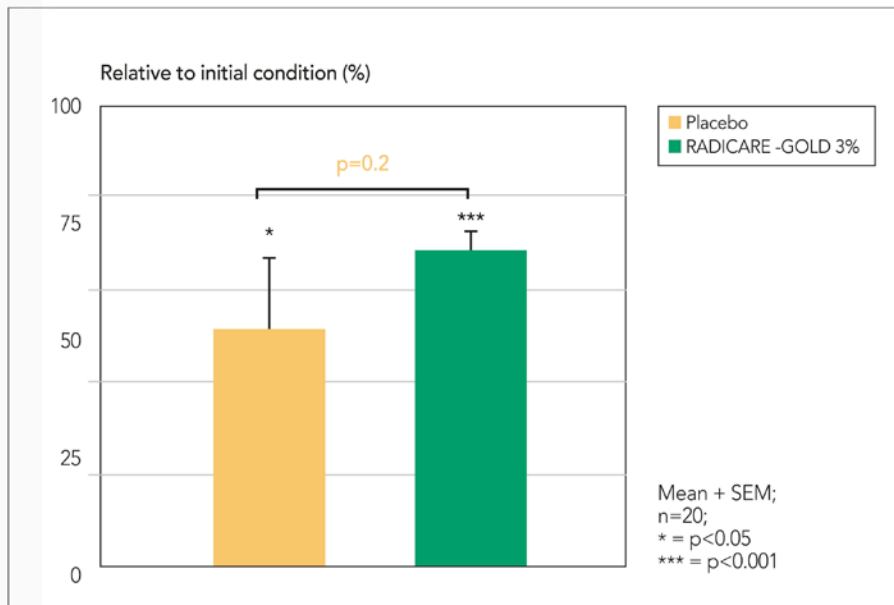


Figure 10: Increase in the epidermal barrier strength on the face. Skin treated with the active showed a significantly increased barrier strength compared to initial condition and outperformed placebo. Two-tailed, paired Student's t-test.

stratum corneum. This results in damage to the skin barrier, increased corneocyte detachment and reduced hydration. At the intracellular level, these forms of radiation can either directly provoke ROS formation or influence this by exciting photosensitisers, which transfer their energy to create ROS. Besides UV and HEV light, other radiation sources in modern technology or simply stress can increase the level of ROS by putting pressure on the respiratory chain in the mitochondrial membrane so that it leaks electrons, which are the main source of intracellular ROS production. Radicare-Gold adds valuable antioxidants in the form of β -carotene and lutein to the skin barrier and the living cells. Both molecules can eliminate ROS at an early stage by neutralising excited molecular states, both in the skin barrier and in living cells, acting synergistically. They prevent leaked electrons combining with atmospheric oxygen at the ROS formation stage, especially in the sensitive biomembranes. In this way, Radicare-Gold provides for a robust skin barrier on the outside but also on the inside, protecting the skin against ROS-induced premature skin ageing provoked by all kinds of radiation. PC

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