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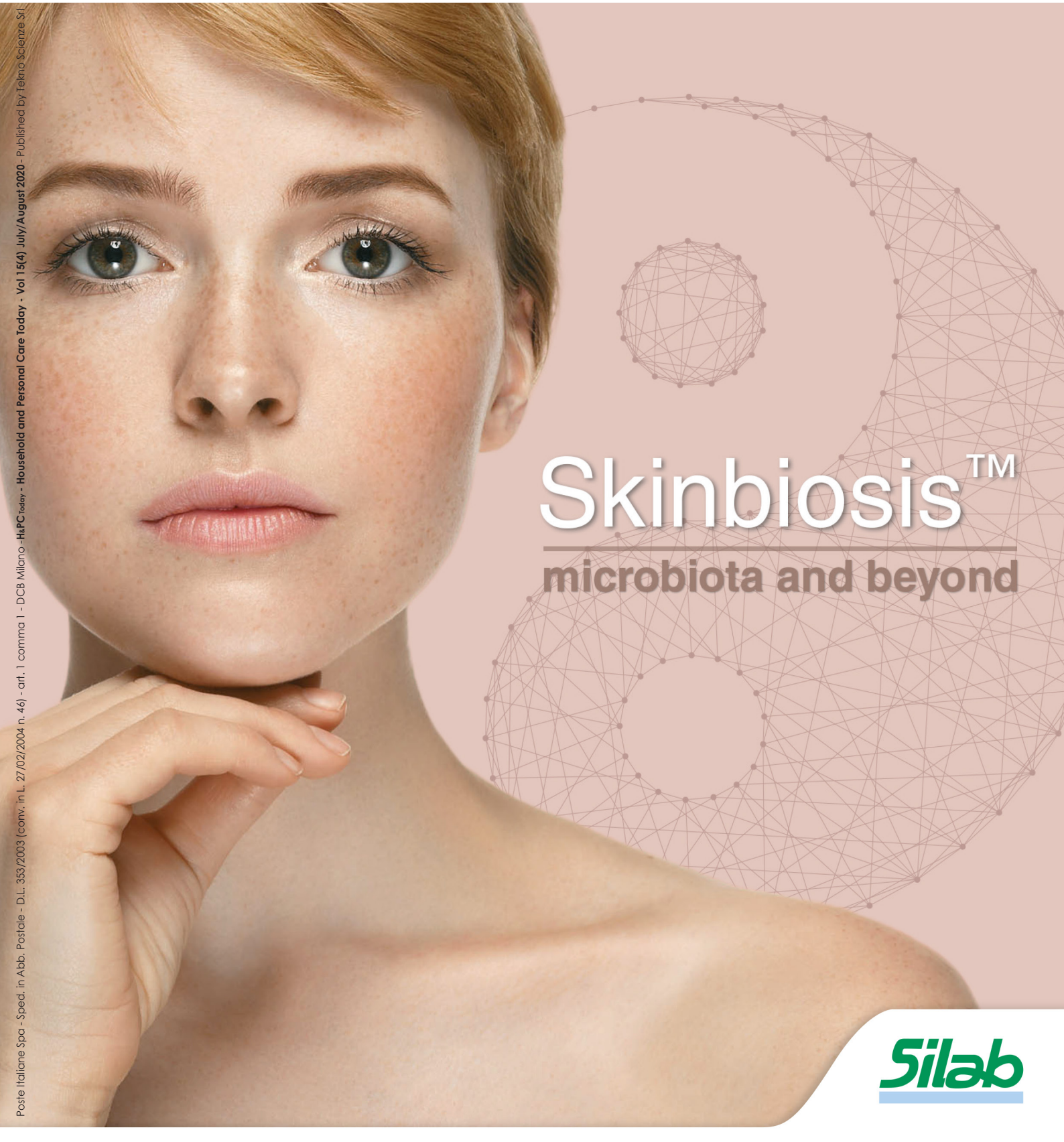

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Epidermal jetlag

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Abstract

We all have a master clock located in our brains. The preprogramed circadian rhythm is activated when our eyes perceive the blue light part of the light spectrum. With the help of certain clock genes such as CLOCK, ARNTL, PER and CRY, our circadian rhythm regulates all the clocks of our body and its organs. If this rhythm is disturbed, what occurs is similar to jet lag. Even our skin suffers from jetlag induced by long distance travel, long working days, usage of blue light-emitting media or UV light exposure that causes additional damage to the skin barrier and cellular DNA.

We here describe the best way to recalibrate the biorhythm of our skin thanks to two powerful circadian antioxidants (1) obtained from rice and rosemary. By supporting the master genes of the epidermal clock, the cosmetic active ingredient (CE) immediately strengthens the skin barrier, increases moisture and eliminates redness.

Keywords

- Jet lag
- Circadian rhythm
- Clock genes
- Skin protection
- Modern life
- Skin care
- Peer Reviewed

INTRODUCTION

The circadian biorhythm

Since life developed on Earth, it has always had to deal with the day and night cycle and already the earliest life forms even developed strategies to benefit from this (2). Motile algae, for example position themselves closer to the water surface to optimize their photosynthesis and sink down in the night to recover from light stress (3). So they, in effect, sleep, as does every higher life form in a defined biorhythm, called the circadian rhythm. The term circadian is derived from the Latin words circa and dies, which translates as "about a day".

It is the sun that controls the behaviour of all creatures that have a diurnal lifestyle. As soon as sunlight hits the eye, the brain of diurnal animals and humans will stop the secretion of the sleep hormone melatonin. Instead, daytime hormones like serotonin, adrenalin, cortisol and testosterone are released. Blood pressure rises and we become active. When the sun sets and it becomes dark, melatonin secretion starts and we become tired. All daytime processes are slowed down and regeneration starts (4).

These biological processes have been optimized over many millions years of evolution so that they have become preserved in our DNA. It is such an influential part of us that we experience major jet lag when we travel over several time zones or if we party until sunrise. In our modern world, we constantly run counter to this pre-programmed cycle as we tend to be awake several hours after dawn, completely ignoring the effective day and night time. Watching TV or reading on tablets and smart phones even aggravate the situation as blue light hits our eyes which, as a result, activates the "daytime program" of our body. All these unnatural habits lead to severe disturbance of the circadian rhythm (5) and also impact on our skin.

Genes regulate the circadian rhythm

Why is the circadian program so powerful? The reason is gene activation. During evolution, a set of crucial genes evolved that are switched on in a 24-hour rhythm, triggered by daylight. The two most important daytime regulators are the CLOCK and ARNTL (formerly known as BMAL1) proteins (Figure 1) [5]. They form an activator complex that switches on a large battery of genes needed for our daily activity [6]. Among these are genes controlling hormone secretion, metabolic activity, DNA repair and the cell cycle but also PER1-3 (period proteins) and CRY1/2 (cryptochrome proteins), natural antagonists of the CLOCK/ARNTL activator complex. When PER and CRY proteins accumulate, they bind and remove the activator complex from the daytime genes and switch them off. Due to their short half-life they degrade overnight, allowing CLOCK and ARNTL to activate gene expression on the following morning. The circle restarts.

In our body, we have a master clock that employs precisely this mechanism (7). It is located in the suprachiasmatic nucleus (SCN) of the brain and is activated by light signals coming from the eyes. To clock in peripheral tissues, hormones are secreted, synchronising all cells in the body, including the skin cells (8).

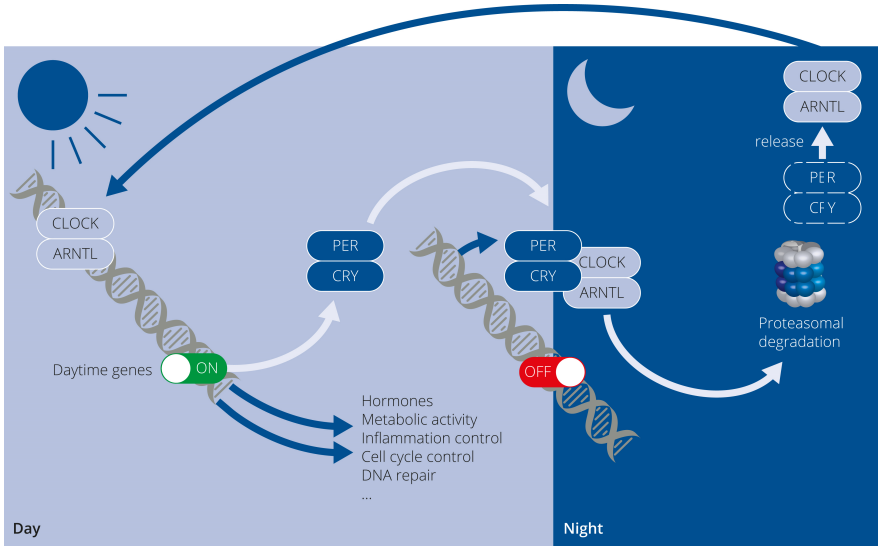

Figure 1. Circadian clock gene regulation. CLOCK and ARNTL proteins bind to target DNA sequences and switch on daytime activity genes, Accumulation of the natural antagonists PER and CRY switch off gene expression. Further information in the text.

Impact on the epidermal clock

To maintain its functionality, the skin permanently sustains homeostatic processes of assembly and disassembly. These processes are true mainly for the dermis, while the epidermis continuously renews itself within each 3-4 week period. The basal layer of keratinocytes consists of stem cells that divide to create daughters that are pushed outward to serve as one of the tightest tissues our body can produce: the stratum spinosum of the epidermis. The further fate of the cells is to differentiate into granulocytes, which provide the stratum corneum with the molecules required to build an effective skin barrier, mainly lipids and natural moisturisation factor (NMF). Their cell envelopes later on serve as corneocytes, which are shed from the skin (9).

What may seem to be like an assembly line process can also be described as a recurrent mechanism that occurs on a day-to-day basis. Here, the epidermal clock plays an essential role (Figure 2) (10).

In the morning, the cycle starts with the activation of the circadian clock genes and the skin barrier starts to recover from the stress it was exposed to the day before. The biggest threat in daytime is exposure to the sun and consequently to UV radiation (11). To counteract this, light-induced and ROS-mediated DNA damage is steadily repaired during daytime (12), which increases the inflammatory status of the skin and produces skin reddening. Nevertheless, in the afternoon, the skin barrier reaches its maximum resistance to wind and weather and also skin hydration reaches its maximum as the cells of the stratum granulosum reach final differentiation (13). When we fall asleep, the skin can recover and it starts to create a new layer of the epidermis by means of stem cell division in the stratum basale. Metabolically-induced ROS are efficiently scavenged and ROS-induced DNA damage is repaired by carefully clock-set repair enzymes (14). The most common oxidative DNA lesion is 8-oxoguanine, which has a major potential to create permanent mutation in the next cell division cycle (15). Hence, enzymes such as 8-oxoguanin DNA glycosylase (OGG1) are upregulated and reach their maximum concentration during the night (16). Having the lowest level of skin hydration and weakest skin barrier at that time (17) can cause itching but normally this has no effect on us because we are asleep. Decreased epidermal stress is visible in the form of reduced skin redness in the morning and the cycle restarts. Thanks to this mechanism, we have the highest skin protection capacity when we need it most and the weakest when we neither need it nor are aware of it.

The biggest risk: UV radiation

Although the largest fraction of UV light from the sun is absorbed by the atmosphere, a significant amount hits our skin and causes damage (18). It can directly damage the DNA or induce the formation of reactive oxygen species (ROS) which eventually damage the DNA of the skin cells and accelerate cellular senescence.
In addition, UV radiation has a negative impact on the circadian clock genes (19) and can cause "cellular jetlag". Powerful sunscreen molecules and anti-oxidants can protect the skin against an excessive UV burden, keep the internal clocks in line and stabilize the skin's functions.

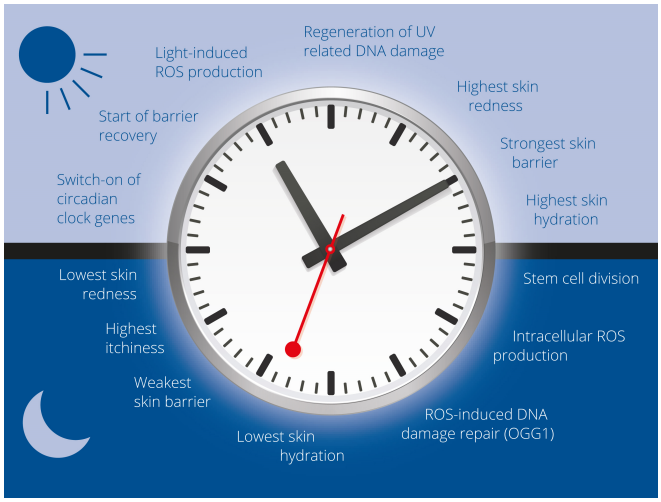

Figure 2. The circadian cycle of the epidermis. During daytime, the epidermal cells are exposed to numerous deleterious factors of which UV light is the most harmful. The cells need to recover and undertake repairs to damage during the day and especially at night, resulting in disturbance of the skin barrier function and low moisture content particularly during the latter. The timing of the epidermal clock leads to full recovery of the skin barrier during daytime.

Aim of the studies

We wanted to evaluate, whether cosmetic formulations have an impact on skin parameters changing throughout the day. As some of them might be regulated by circadian gene expression or hormone fluctuations, we investigated the effects of a cosmetic formulation with and without an active ingredient on physical skin parameters at two times of the day. Furthermore, we tested the active ingredient for an influence on gene expression of essential genes of the circadian clock in human keratinocytes when stressed with UV-light.

MATERIAL AND METHODS

CELLIGENT® (INCI: Helianthus Annuus (Sunflower) Seed Oil, Ethyl Ferulate, Rosmarinus Officinalis (Rosemary) Leaf Extract, Tocopherol) was used as the active ingredient in the performed studies. Its active ingredients are carnosic acid derived from rosemary and ethyl ferulate derived from rice. Rosemary (Rosmarinus officinalis) is an evergreen shrub with an intensely aromatic scent originating from the Mediterranean region. Around 90 % of its antioxidative properties are ascribed to carnosic acid, which is one of the most powerful anti-oxidants (20). Ethyl ferulate is a natural antioxidant with UV-absorbing properties. It neutralizes oxygen radicals which have been generated as a result of exposure to UV radiation and thus protects cells against oxidative stress (21-23). Ethyl ferulate is produced from ferulic acid obtained from rice, which is one of the plants studied in most detail when it comes to the circadian rhythm of crop plants (24). Due to this composition (carnosic acid, ethyl ferulate), the active ingredient is termed "CE" throughout this publication.

In-vitro study

Primary human keratinocytes from a 37-year-old female donor were cultivated in a 12 h light/dark environment.

A synchronized induction of the circadian rhythm was achieved by means of supplementation with 100 nM dexamethasone for 20 minutes (25). Afterwards, the cells were irradiated with 15 mJ/cm² UVB. The simulated day/night cycle was maintained over 2 days and samples were taken after 6, 18, 30 and 42 hours. It was not expected that the light/dark cycle has a major contribution on the gene expression as the circadian gene expression was induced by dexamethasone and the central, light induced steering mechanism from the brain is missing. Gene expression was determined using qRT-PCR with technical duplicates on n = 3 samples. Addition of active ingredient depended on results of a cell viability assay in each experiment.

In-vivo study

The in-vivo study was performed in accordance with the principles of good laboratory practice (GLP) and good clinical practice (GCP) and in compliance with the quality assurance system requirements. Studies were conducted with respect to World Medical Association in the Declaration of Helsinki. All study participants signed a written informed consent at the beginning of the study. In this study, skin hydration, transepidermal water loss (TEWL) and skin redness were assessed. All test parameters were initially measured before application of any formulation at day 0 in the morning (between 8:00 and 10:00) and in the evening (between 20:00 and 22:00) for baseline determination. Subsequent measurements on days 28, 56 and 84 were performed prior to the application of the corresponding formulation. Half of the subjects applied placebo while the other half applied the identical formulation containing 3 % CE. Skin hydration was measured with a Corneometer® CM 825 at three different positions on the forehead. TEWL was assessed with a Tewameter® TM300 in the central region of the forehead. For determination of skin redness, measurements with a Mexameter® MX 18 in the malar region were performed.

Statistics

Unpaired Student's t-tests were performed on all shown parameters. The statistical values in black are the result of comparison with baseline status while the blue values are the result of comparison with placebo or vehicle control.

RESULTS

In vitro study

The circadian clock was induced in primary keratinocytes (see material and methods). While ARNTL exhibited pronounced circadian regulation, the CLOCK gene was not significantly regulated in all tested conditions (Figure 3, dark blue lines). Without an external trigger, CE did not significantly influence the expression of circadian genes (not shown), demonstrating its good compatibility with epidermal skin cells.

Irradiation of the keratinocytes with UVB light extensively affected expression of the ARNTL and CLOCK genes after 6 hours in the daylight phase and disrupted the circadian cycle. Gene expression recovered after 18 hours in the dark phase and then reached normal levels in the vehicle control experiment. In contrast, cells treated with 0.01 % CE upregulated gene expression faster, which after 30 hours reached a maximum in the daylight phase of treatment day 2, with values for CLOCK and ARNTL roughly twice that in the vehicle control. Importantly, there was no effect on the periodicity of gene expression.

As a result, the positive regulation of the circadian clock genes should result in a positive regulation of downstream genes. An important gene among these is the DNA repair gene OGG1, coded for 8-oxoguanine DNA glycosylase. This enzyme repairs DNA damage due to internal oxidative stress but also induced by UVB irradiation. Indeed, incubation with just 0.001 % CE resulted in recovery of the suppressed gene expression of OGG1 after 18 hours with outperformance compared to vehicle control after 42 hours (Figure 4). The peak was reached in the dark, the time of most effective oxidative DNA damage repair. This indicates an increased DNA repair capability after UVB irradiation in active-treated cells.

As this in-vitro experiment is just the result of investigations on one donor, repeating the experiment with donors from different ages and ethnicities should be performed to confirm this initial finding.

Figure 3. ARNTL and CLOCK gene expression fall after exposure to UVB radiation. The expression of both daylight-active genes was heavily affected after UVB irradiation but upregulated to a level almost twice than that in vehicle control 30 hours after UVB irradiation in the presence of 0.01 % CE.

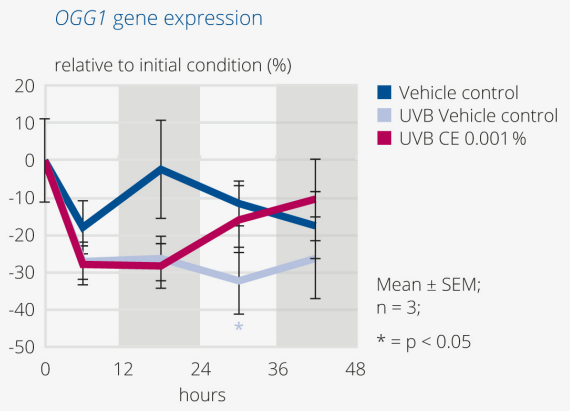

Figure 4. CE restores DNA repair after UVB irradiation. From 18 hours after irradiation, the gene expression of the 8-oxoguanine repair gene OGG1 recovered back to normal levels in the presence of CE while the OGG1 expression in vehicle control remained significantly reduced.

***In vivo* study**

Cosmetic formulations interact with the environment and can generate an extrinsic ROS burden on the skin barrier. Light stress during the day can lead to skin reddening in the evening due to pre-stages of sunburn or other stress factors. Our objective was to determine whether application of a cosmetic formulation with CE influences basic skin parameters and how these relate to the skin's own circadian rhythm. To investigate the skin's baseline circadian rhythm and on treatment with cosmetic formulations we performed a study on 43 subjects with healthy, Caucasian skin aged 35 – 65 years. The formulations were applied twice daily on the face for 84 days.

General development of test parameters throughout the study:

Skin hydration: As expected, skin hydration with placebo and 3 % CE were significantly improved at every measurement time point ($p < 0.001$) by up to 40 % over baseline. CE was up to 11 % significantly superior over placebo (not shown).

Barrier strength was significantly improved as the TEWL values dropped by up to 26 % in the evening on day 56 compared to placebo (Figure 5). The morning value was almost the same. Application of CE improved the barrier strength in the same way during the day and the night. While placebo did not significantly improve TEWL in the morning measurement compared with baseline status, it actually significantly worsened in the evening measurement by up to 15 % (not shown).

Skin redness during the evening measurement was significantly reduced over baseline (day 28) and placebo (days 28, 56, Figure 6). Interestingly, placebo redness values were increased by up to a significant 14 % over baseline ($p < 0.001$, not shown). Also the morning redness in subjects applying CE decreased compared to placebo, however not significantly. The positive effect was also maintained after 84 days (not shown).

Circadian changes at baseline:

Before treatment, the skin parameters hydration, TEWL and redness were assessed in the morning and in the evening. We observed a circadian regulation of skin moisture, which was low in the morning and significantly higher in the evening.

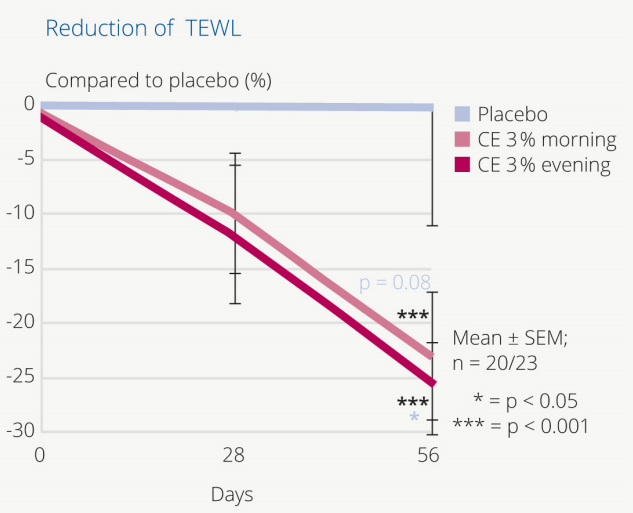

Figure 5. CE is an excellent barrier-strengthening agent. It reduced TEWL in the morning as well as in the evening. Placebo values were set to "0".

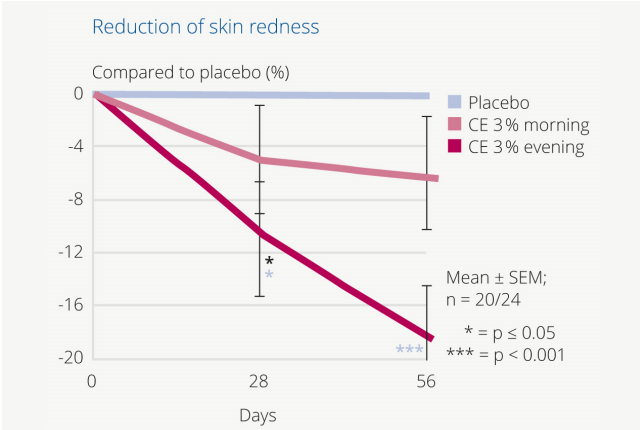

Figure 6. Skin redness continuously falls in the morning and evening measurements compared to placebo. The effect was significant over placebo and baseline in the evening. Placebo values were set to "0".

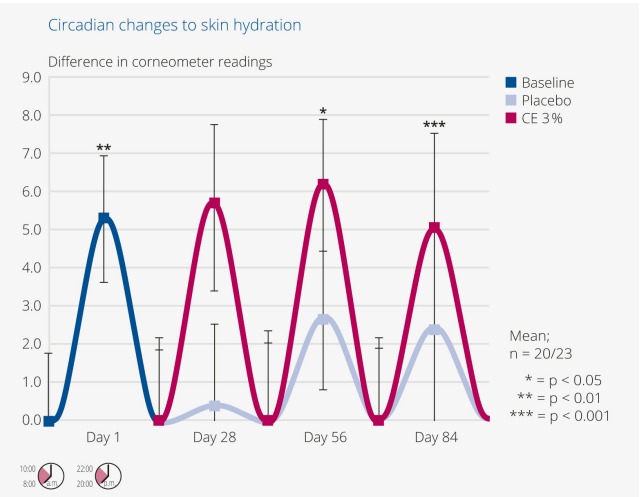

Figure 7. Circadian changes to skin hydration. The baseline values of the entire study population revealed a significant increase in skin moisture in the evening (blue line) compared to the morning value. Application of CE preserved this periodicity while placebo interfered with this process. Statistical values indicate significant differences between the morning and evening measurements at each measurement day.

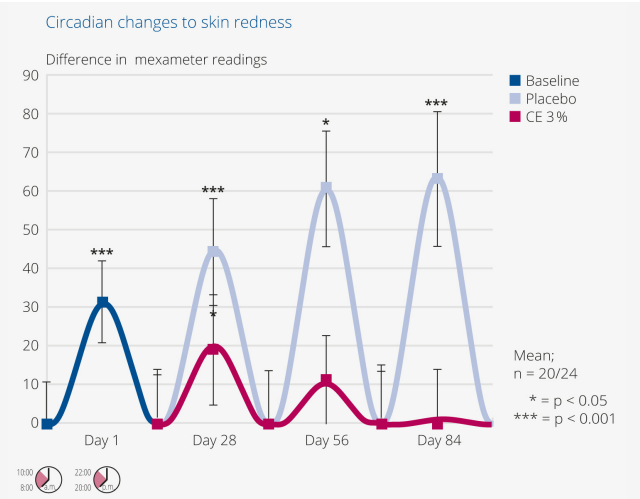

Figure 8. Circadian changes to skin redness. The facial skin redness was significantly higher in the evening at baseline (blue line). While placebo treatment led to a doubling of the amplitude, CE reduced skin reddening during the day up to no detectable difference compared to the morning value.

On the other hand, TEWL was high in the morning and low in the evening; however there was no significant difference. These findings are in agreement with the data from the literature (26).

Skin redness developed during the day and was significantly higher in the evening than in the morning. We postulate that this is more likely to be an extrinsic process evoked by sunlight-induced inflammatory reactions rather than an intrinsically-induced process dependent e.g. on blood pressure (27). To support skin function, maintenance of the circadian rhythm of skin hydration and TEWL needs to be preserved while skin redness, as an extrinsic process, should be reduced to promote a non-inflamm'ageing environment.

Circadian changes during test formulation application

The baseline measurements revealed a significant circadian variability only for skin hydration and skin redness. Both parameters were evaluated for their circadian development throughout the entire study duration of 84 days.

Skin hydration: Investigation of the circadian differences at day 28, 56 and 84 after test formulation application revealed a significant maintenance of the circadian skin moisture changes throughout the application of CE. Figure 7 shows the difference of the morning values in comparison with the evening values on each measurement day, irrespective of the overall improvement by 40 % for the active formulation. In contrast, the variation in skin moisture throughout application with placebo dropped dramatically to a non-significant level as expected for a suppressed circadian rhythm in aged skin. This indicates that the natural intrinsic circadian regulation of skin moisture was disturbed in the presence of the placebo formulation. On the other hand, the amplitude was preserved with the CE-containing formulation, reflecting the behaviour of youthful skin.

Skin redness: The amplitude of skin redness increased during the study in the case of placebo application (Figure 8). At day 84, the amplitude doubled, which means that skin redness was even more pronounced in the evening compared to the morning value. In contrast, CE continuously reduced the amplitude to zero, which means the skin redness was identical in the morning and evening measurements at day 84. As skin redness is not an intrinsically circadian regulated process, we can assume that the activity of CE protected the skin during the daytime from deleterious light-induced inflammatory reactions.

SUMMARY

Some skin parameters change within the 24 hour cycle of the day. Here, we could show that cosmetic formulations can interfere with the natural fluctuations on skin hydration and skin redness. Incorporation of an active ingredient was able to preserve the natural skin hydration fluctuation, possibly by positively regulating genes responsible for maintaining the proper circadian rhythm in skin cells and facilitate proper circadian regeneration of the skin. In contrast, skin redness was decreased and reached the same evening level compared to the morning level after 3 months. As skin reddening is a result of increased microcirculation, caused mainly by extrinsic effects, the result is not a consequence of interfering with the circadian rhythm but driven by anti-oxidative effects reported elsewhere (1).

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