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Fixing age with lipids:
Improvement of the epidermal barrier function in mature skin

KEYWORDS: Liver X receptor, epidermal lipids, mature skin, anti-ageing.

Abstract Epidermal lipids, among other components, constitute the seal for the outermost skin layers and the glue for the corneocytes. Epidermal lipids, however, are reduced in mature skin and may represent the underlying cause of increased susceptibility, diminished capacity to recover, and chronic dryness of mature skin. Hence, reactivating epidermal lipid synthesis represents a promising anti-ageing strategy for mature skin.
Earlier in-vitro experiments implied that a cosmetic active ingredient, based on a Gynostemma pentaphyllum extract, reactivates lipid synthesis via the Liver X receptor (LXR). Here we show that the reactivation of lipid synthesis may indeed translate into improved barrier integrity and repair.
The skin barrier was disturbed by tape stripping. Subsequent placebo application for 28 days only minimally regenerated the barrier whereas the cosmetic active imparted full regeneration. Barrier strengthening was also supported by reduced corneocyte detachment. Taken together, our data suggest that cosmetic actives increasing the amount of skin lipids may indeed help to replenish the skin from within. This will eventually improve typical signs of mature skin such as barrier deficiencies, but also chronic dryness and wrinkles

INTRODUCTION

The barrier integrity of mature skin is disturbed

Mature skin, as a consequence of intrinsic and extrinsic ageing (1), is associated with weaknesses of the skin barrier: The corneocytes of the stratum corneum are morphologically changed and the synthesis of epidermal lipids only functions to a limited extent. The spaces between the cells are no longer properly sealed by the intercellular lipid lamellae (2). This eventually leads to an increased permeability of the skin barrier; the skin becomes more susceptible to external aggressors, and water can evaporate from the skin more quickly. However, functional abnormalities often become evident only when the tissue is either stressed or required to carry out repair processes. To this end, the aged epidermis is perturbed much faster and recovers much more slowly in comparison to young skin (3). For example, resistance to sequential tape stripping was markedly reduced in aged epidermis: 18 versus 31 strippings were required to disturb the epidermis in old versus young skin (3).
In essence, this dysfunction can, at least in part, be attributed to a global deficiency in all the key stratum corneum lipids, resulting in decreased lipid layers in the stratum corneum interstices. Notably, on average, 80-year-old skin contains 65% fewer epidermal lipids than 25-year-old skin (4). The reduced lipid content may also contribute to the commonly dry and xerotic eczema skin of the aged.

The role of LXR in epidermal lipid biology

The liver X receptor (LXR), a nuclear receptor, is an important regulator of cholesterol, fatty acid and glucose homeostasis. LXR is expressed in keratinocytes and fibroblasts of the skin (5). There is increasing evidence that LXR activators also have remarkable effects on epidermal biology (Figure 1):

1. Keratinocyte differentiation is a sequential process, which ultimately results in the formation of the stratum corneum, an epidermal layer consisting of corneocytes surrounded by a lipid-enriched extracellular matrix. Corneocytes provide strength due to extensive transglutaminase-mediated cross-linking of proteins such as loricrin or involucrin to form the cornified envelope. Treatment with LXR activators stimulates an increase in the markers of keratinocyte differentiation; i.e. transglutaminase, involucrin (5).

2. In addition, the corneocytes provide a scaffold which is required for organising the extracellular lipids into lamellar membranes. These extracellular lipid layers are generated and maintained by four key steps:
   - epidermal lipid synthesis,
   - lamellar body formation,
   - lamellar body secretion, and
   - extracellular processing of precursor lipids to lipids, which form the extracellular lipid membranes.

Studies have shown that LXR activators activate all of these key steps (5).

3. Finally, activation of LXR can suppress cutaneous inflammation by inhibiting the production of pro-inflammatory cytokines (5).

As a consequence, LXR activators could improve barrier homeostasis by affecting a number of the key steps required for the formation of the extracellular lamellar membranes which mediate the permeability barrier (references (6-8) comprehensively review the permeability barrier) . Indeed, recent studies have shown that LXR activators accelerate the recovery of the permeability barrier following acute barrier disruption (9). Because of their broad profile of beneficial effects on skin homeostasis, LXR activators represent ideal candidates for a holistic anti-ageing concept against chronological skin ageing (10); a readjustment of the lipid metabolism is expected to have beneficial impact on the regenerative capacity, firmness, elasticity, hydration and roughness of the skin.

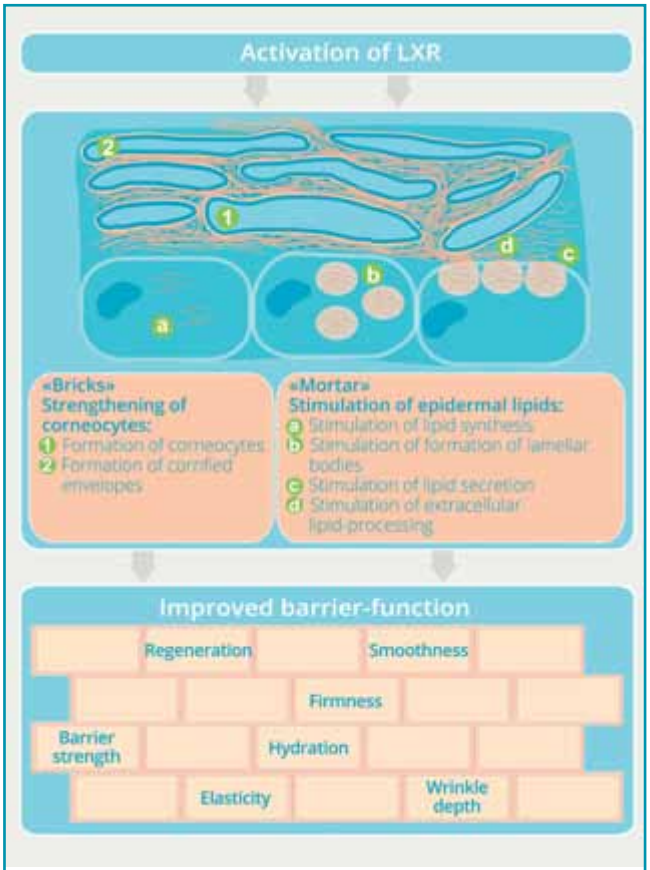

Figure 1. LXR activation and its positive effects on skin barrier function.

***G. pentaphyllum* activates LXR and thereby stimulates the key enzymes of epidermal lipid synthesis**

Gynostemma pentaphyllum, also known as immortality herb is an adaptogenic plant with an extensive active profile (11). The main active ingredients were identified as so-called gynosaponins or gypenosides, a particular group of saponins which greatly resemble the saponins contained in the ginseng root.

A recent study has revealed that some of these gynosaponins exert an activating effect on the Liver X receptor (12).
Based on this finding, the cosmetic active ingredient REFORCYL® (hereafter referred to as active ingredient) has been developed. The active ingredient contains a combination of extracts from *Gynostemma pentaphyllum* and from *Cistus incanus* (INCI: Glycerin, Aqua, Glutamine, Decyl Glucoside, Phenethyl Alcohol, Citric Acid, Cistus Incanus Flower/Leaf/Stem Extract, Gynostemma Pentaphyllum Leaf/Stem Extract). The latter was added to strengthen the reduced antioxidative defence system found in mature skin.

We hypothesize that the combination of a plant-derived LXR-activator and a plant extract with antioxidative efficacy represents a very promising anti-ageing strategy for mature skin. Notably, we already corroborated that in particular *Gynostemma pentaphyllum* extract activates LXR and its downstream genes *in-vitro*. We also demonstrated that the application of the active ingredient measurably, perceivably and visibly improves the condition of mature skin as it improved skin hydration, firmness, elasticity, smoothness, and wrinkle depth (13-15).

Now, after having established that *Gynostemma pentaphyllum* extract activates LXR *in-vitro* (12, 13) and that the active ingredient provides *in-vivo* skin benefits such as wrinkle reduction (14, 15), we here aimed to provide the missing experimental link that LXR-activation indeed entails increased epidermal barrier function. We postulated that (re)activated epidermal lipid synthesis would translate into barrier strengthening effects which, in turn, would become evident by *e.g.*:

1. Reduced corneocyte detachment, and
2. Improved barrier integrity and repair

EXPERIMENTAL SECTION

Providing the glue to seal the epidermal barrier (*in-vivo* study)
Rationale: Skin lipids, among other things such as corneodesmosomes, are responsible for holding corneocytes together and for providing cohesion (brick and mortar model, Figure 1): the more lipids present, the fewer corneocytes are expected to detach to adhesive tape. To this end, Corneofix® tape and a Visioscan VC 98 with its corresponding software were used to quantify corneocyte detachment (dequamation index, Figures 2 and 3) and the better the quality of the barrier, the lower the detachment values. In order to get access to the deeper layers of the stratum corneum, skin areas were pre-stripped using one or two TESA® strips.

Skin lipids are also thought to be responsible for sealing the spaces between the corneocytes and for preventing water evaporation: the more lipids there are, the lower the transepidermal water loss (TEWL). To this end, a Tewameter® TM 300 was used to assess the TEWL and barrier integrity. The barrier repair was ascertained by measuring the TEWL before and after artificially damaging the skin with one or two TESA® strips - the better the barrier and the recovery, the lower the TEWL values.

St	Substance	INCI Name USA	% [w/w]	Manufacturer
1	Water demin.	Water	91.90	several
	Euxyl PE 9010	Phenoxyethanol, Ethylhexylglycerin	1.00	Schuelke & Mayr, DE
2	Keltrol CG-SFT	Xanthan Gum	0.20	CP Kelco, US
	Carbopol ETD 2020	Acrylates/C10-30 Alkyl Acrylate Crosspolymer	0.40	Lubrizol, US
3	Cetiol OE	Dicaprylyl Ether	2.50	BASF, DE
4	NaOH solution 10%	Sodium Hydroxide, Water	1.00	several
5	REFORCYL®	Glycerin, Water, Glutamine, Decyl Glucoside, Phenethyl Alcohol, Citric Acid, Cistus Incanus Flower/Leaf/Stem Extract, Gynostemma Pentaphyllum Leaf/Stem Extract	3.00	RAHN AG, CH

Table 1. Test formulation. An equivalent formulation withouth REFORCYL® served as placebo.

Implementation: Double-blind, placebo-controlled and randomised study design: 10 female volunteers with Caucasian skin and presenting a mature skin typology (i.e. dry, dull, thin) took part in the study. The age range was 46 to 58 years (average 52.2 years). On one forearm, a cream containing 3% active ingredient was applied twice daily by the volunteers at home over a period of four weeks (the formulation is shown in Table 1). Placebo, i.e. the same formulation but without active ingredient, was applied on the other arm.

Each arm contained two test areas: one area was stripped once with TESA® strips, whereas the other area was stripped twice (single- and double-stripped areas). TESA® stripping was done before and after the 28-day application period. Corneocyte detachment was measured just after the TESA® stripping, and the TEWL was measured before and immediately after TESA® stripping.

RESULTS AND DISCUSSION

Corneocyte detachment

Single-stripped area: After 28 days, the amount of corneocytes that were detached using Corneofix® tape increased by 24% on the placebo-treated areas, whereas it decreased by 14% on the ingredient-treated areas. Or in other words, compared to the beginning of the study fewer corneocytes were removed when the active ingredient was applied for 28 days. This implies that the active ingredient indeed increases the amount of skin lipids (Figures 2 and 3).

Double-stripped area: Likewise, the corneocyte detachment increased by 2.9% on the placebo-treated areas, whereas it decreased by 5.5% on the ingredient-treated areas. However, statistical significance was not reached and it seems that strengthening the barrier becomes more difficult as deeper layers of the skin were reached (Figure 3).

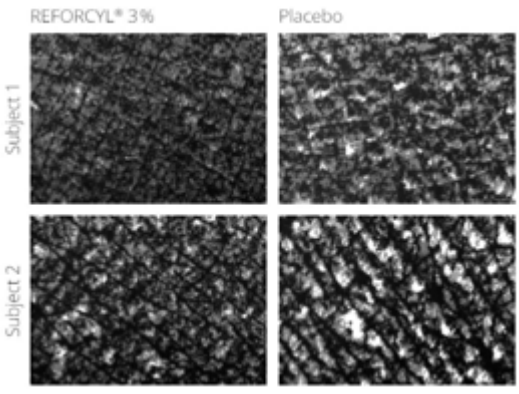

Figure 2. Qualitative reduction of corneocyte detachment. Qualitative illustration of the reduction in corneocyte detachment after 28-day application of 3% active ingredient as compared to placebo. Corneofix® tapes from two volunteers are shown.

Barrier integrity and repair

Single-stripped area: The skin barrier was substantially disturbed and the TEWL increased by about 10% upon stripping. Subsequent application of placebo for 28 days only induced minimal regeneration, and the TEWL remained elevated (+8% compared to day 0). Moreover, a second stripping at the end of the study led to cumulative damage (+20%). The active ingredient, in contrast, not only fully regenerated but even strengthened the barrier: upon application of the active ingredient, the TEWL decreased by 18.3% and was even lower compared to the beginning of the study (-9%). Finally, the active ingredient also prevented cumulative damage upon repetitive disturbance (Figure 4).

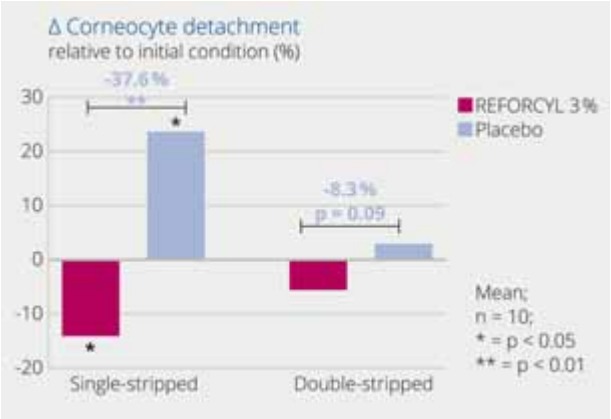

Figure 3. Quantitative reduction of corneocyte detachment. Corneocyte detachment was quantified using Corneofix® tape before and after a 28-day application period of either 3% active ingredient or placebo. The lower values obtained with the active ingredient point to a barrier strengthening effect, since fewer corneocytes became detached after the application period than before. We believe that this is at least in part due to increased amounts of epidermal lipids, which constitute the glue for corneocyte cohesion and adhesion.
In order to obtain access to deeper epidermal layers, single- (left) and double- (right) TESA® stripping was performed and Corneofix® tape was subsequently applied. The statistical values shown in violet relate to the comparison of active ingredient with placebo, whereas the black values relate to the comparison of the respective treatment with the initial condition. Two-tailed, paired t-test.

Double-stripped area: Barrier disturbance was even stronger, as evidenced by an approximately 20% elevated TEWL. It appeared that such substantial disturbance takes longer to disperse: regeneration upon application of placebo was completely absent and the TEWL remained constantly high. Moreover, a second double-stripping at the end of the study led to a doubling of the TEWL (44% compared to day 0).
The active ingredient, in strong contrast, led to significant regeneration and halved cumulative damage upon stripping: Notably, in the ingredient-treated areas the TEWL values after two double-strippings were comparable to those in the placebo treated area after just one double-stripping (Figure 5).

Barrier function of the stratum corneum of human epidermis is facilitated by different means such as lipid synthesis, lamellar body formation, processing of precursor lipids , cross-linking of proteins etc., which apparently can all be optimised by LXR activation (5). Nevertheless, another important factor that affects the function of the skin lipid barrier function is the lateral lipid packaging, i.e. orthorhombic versus hexagonal packaging (16). Our results confirm improved attachment of corneocytes as well as better stratum corneum recovery and suggest a general improvement of barrier function upon treatment with the active ingredient. Solid conclusions concerning lipid synthesis and packaging mainly remain hypothetic and would require further investigations.

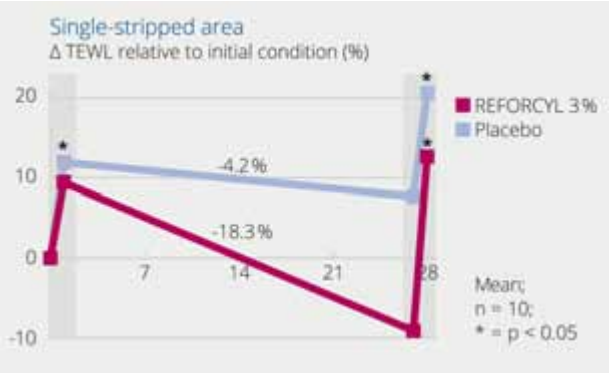

Figure 4. Improvement of barrier repair. The skin barrier was artificially damaged using one TESA® strip before and after a 28-day application period of either 3% active ingredient or placebo. The strongly decreasing TEWL values upon treatment with the active ingredient point to an excellent regeneration efficacy. It was notable that the active ingredient not only completely regenerated the barrier when this was disturbed, but even provided additional barrier strengthening as it even lowered the TEWL below the baseline level. The grey areas indicate TESA® stripping; test products were applied in between. The statistical values relate to the comparison with the initial (baseline) condition. Two-tailed, paired t-test.

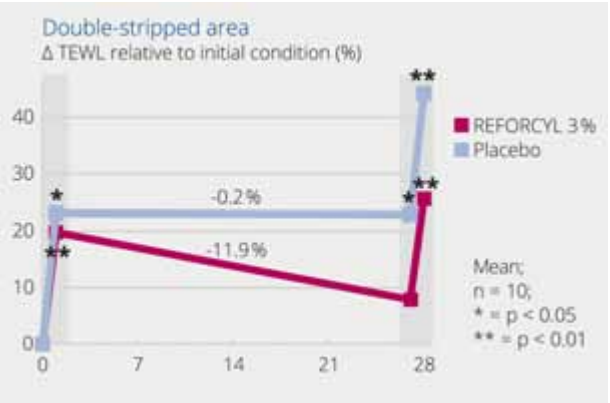

Figure 5. Improvement of barrier integrity. Two TESA® strips were applied. The active ingredient counterbalanced skin barrier perturbations as it provided substantial regeneration and convincingly prevented damage accumulation. For details see Figure 4.

CONCLUSION

LXR is a promising target for the development of potential anti-ageing ingredients as LXR activators modulate multiple pathways that are involved in the origination of skin ageing; e.g. LXR activators induce epidermal lipid synthesis (Figure 1).

Of note, epidermal lipids are severely reduced in mature skin and may represent the underlying cause of increased susceptibility to environmental damage, diminished capacity to recover, as well as chronic dryness of mature skin.

The combination of a *Gynostemma pentaphyllum* and a *Cistus incanus* extract represents an innovative anti-ageing concept for mature skin. Taken together, our studies as shown here and elsewhere (13-15) support the following mode of action: *Cistus incanus* extract strengthens the antioxidant defence system which is reduced in mature skin. The immortality herb *Gynostemma pentaphyllum* activates LXR. The activation of LXR, in turn, leads to an activation of the key enzymes of epidermal lipid synthesis and eventually provides new barrier strength. Finally, increased barrier strength positively affects the typical signs of mature skin such as chronic dryness and wrinkles. Overall, the active ingredient attenuates corneocyte detachment and barrier disruption eventually leading to a sevenfold efficacy against the typical signs of mature skin, i.e.: 1) barrier strength and 2) barrier regeneration, but also 3) hydration, 4) firmness, 5) wrinkle depth, 6) elasticity, and 7) skin roughness.

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