MADE TO REFINE

RONACARE®
LUREMIN®

Surface Solutions
Cosmetics

MERCK

WELLNESS TIME

Wellness is in the air….

Since the pandemic dominates our life, people have become even **more focused on their physical and mental health**

Discerning consumers recognize the link between their **lifestyle** (e.g., lack of sleep, diet, use of digital devices, stress) and **skin health/appearance**[2)]

Wellness-focused facial care is a way to **balance stressful lifestyle and recovery time** - to keep skin appearance attractive

Definition

Wellness …is a state of complete social, emotional and physical well-being[1)] …

In the UK

25 % +

of adults are interested in BPC product concepts that help them improve sleep quality, reduce anxiety or help them relax[3)]

1) WHO; https://www.who.int/healthpromotion/about/HPR%20Glossary_New%20Terms.pdf
2) Mintel: A year of innovation in facial skincare, 2021
3) Lightspeed/Mintel; Base: UK: 1,000 internet users aged 16+

CONSCIOUS BEAUTY

A Pillar of the Wellness Mindset

- **Natural ingredients** have been used to remedy skin problems and general health issues since time immemorial
- Today's beauty consumers still rely on natural ingredients – often perceived as being gentle on the skin and on the environment; they like to revisit traditional beauty recipes
- However, since sustainability has become a primary concern, it is essential to **balance biodiversity** and **production technology** so that future generations can enjoy the best in **sustainable cosmetics**

Trustworthiness, transparency, responsibility are at the core of our aspiration to **live well** and **feel good**

SKIN CARE PROMISES

RONACARE® LUREMIN®

Nature-identical. Sustainable. For well-aging.

WELLNESS BRACED BY CARE ROUTINE

- Daily application of a care that satisfies **preventive and protective skin's needs** positively supports emotional stability and well-being
- **Sensing harmony -** with every single use of skin care

NATURAL & SUSTAINABLE INSPIRATION

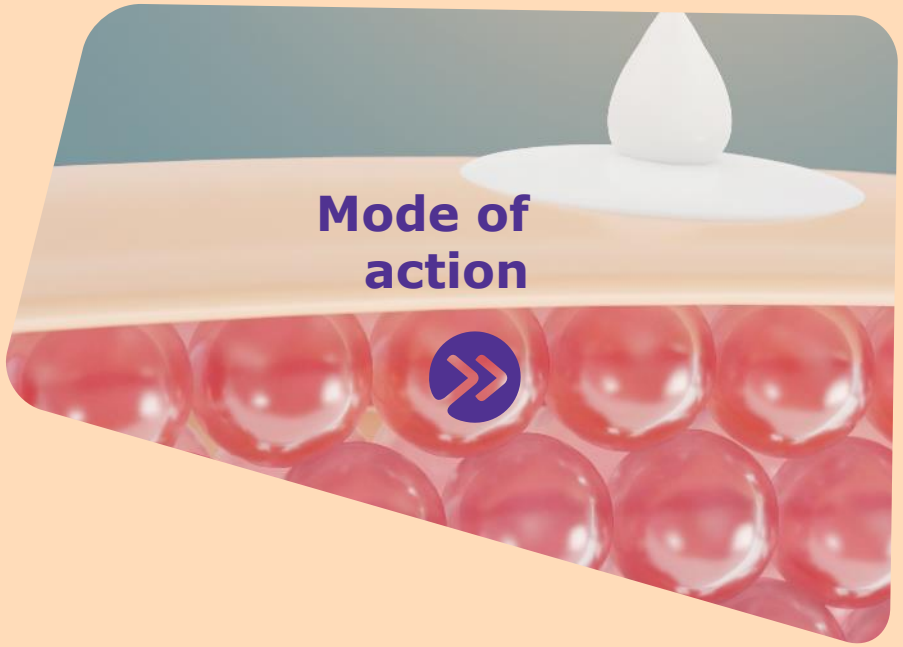
- Translating natural solutions into skin benefits
- Using production expertise to achieve carbon neutrality and create nature-identical solutions

SKIN CARE EMPOWERED BY NATURE

RonaCare® Luremin® is a **nature-identical,** multifunctional active ingredient based on dihydroxymethylchromone (DHMC), a potent phytocompound found e.g., in medicinal rhubarb

- Working on **3 levels** to combat the signs of age- and stress-induced skin aging "inflammaging" – for a **naturally youthful** appearance
- Readily biodegradable, its production process has been carefully optimized to **minimize the carbon footprint – sustainability driven**

List of content



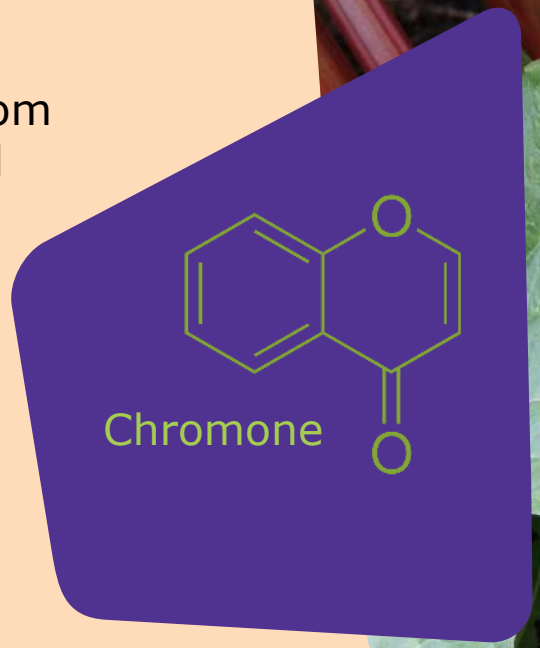
01

RonaCare® Luremin®

INSPIRATION & ORIGIN

CHROMONES IN NATURE

- **Chromones** are naturally occurring phenolic compounds, ubiquitous in the plant kingdom
- They represent one of these chemical weapons used by plants to protect themselves from e.g., pathogens or UV radiation[1)]
- Chromones are also present in healthy human diet
- To date, thousands of chromone derivatives, both from **natural** and **synthetic** origin, have been discovered
- This class of compounds is mainly associated with anti-oxidant, antimicrobial, anticancer and anti-inflammatory activities[2)]



1) Silva, C.F.M. et al. Expert Opinion on Drug Discovery (2018) 13:9, 795-798
2) Semwal, R.B. et al. Phytochem Rev (2020) 19, 761–785

THE CARROT CASE

- Plants are complex organisms that produce different metabolites responding to the environment they live in
- A **carrot under stress** through e.g., ethylene gas or fungi stress, secrets **DHMC (dihydroxymethylchromone)**[1)] and further **chromone phytoalexins**[2)] – to protect against degrading mechanisms
- An attractive approach for cosmetics! Applied to the skin, **phytomolecules** may interact with skin cells and balance the skin's well-being and appearance

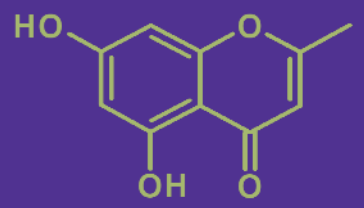
1) Sarkar, S.K. et al. Physiol Plant (1974) 30, 72-76
2) Robeson, D.J. et al. Phytochemistry (1980) 19, 2171-2173

INSPIRED BY NATURE

RonaCare® Luremin®
INCI: Sorbitol, Dihydroxy Methylchromone

- Comprises the naturally occurring active ingredient **dihydroxymethylchromone** (DHMC), also known as **noreugenin**
- Noreugenin was first isolated from the Japanese plant *Nauclea orientalis*[1)]
- It can be also found in numerous **medicinal plants** everywhere in the world; e.g., it has been characterized in *Angelica polymorpha* (China), *Stevia purpurea* (Mexico) or *Rheum emodi* (Indian Rhubarb)

Structure of dihydroxymethyl-chromone (DHMC)



1) Fujita, E. et al. Pharm. Bull. (1967) 15:11, 1682-1686

SUSTAINABLE PRODUCTION

Dihydroxy Methylchromone (DHMC)

- Challenging extraction from plants: low abundance, presence of similar compounds which makes isolation difficult
- Therefore, development of a **more sustainable** synthetic process
  - with optimized use of reaction materials
  - with **water consumption optimization**
  - and eliminating **CO_2 producing steps**
- Obtention of the desired compound in **high yield and purity**

Sorbitol

- Used as carrier to facilitate the dispersion of DHMC in formulation
- Produced from maize/wheat grown in Europe

RonaCare® Luremin®

Nature-identical, sustainable and safe – satisfying the demands of Clean Beauty

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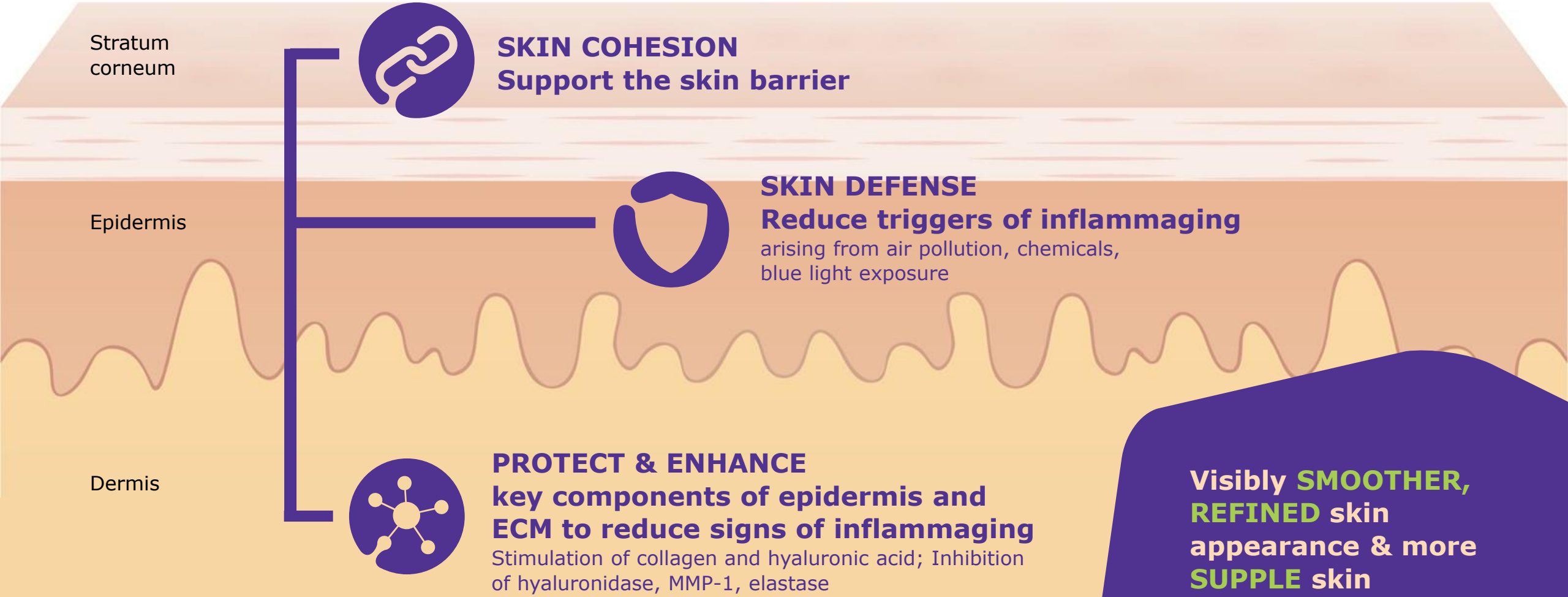
02

RonaCare® Luremin®

PRODUCT FEATURES

Support – Reduce – Protect & Enhance
The 3-level working power of RonaCare® Luremin®



SKIN COHESION
Support the skin barrier

SKIN DEFENSE
Reduce triggers of inflammaging
arising from air pollution, chemicals, blue light exposure

PROTECT & ENHANCE
key components of epidermis and ECM to reduce signs of inflammaging
Stimulation of collagen and hyaluronic acid; Inhibition of hyaluronidase, MMP-1, elastase

Visibly SMOOTHER, REFINED skin appearance & more SUPPLE skin

RonaCare® Luremin®
Performance

1 **Skin cohesion & Skin defense**
Increased skin resistance to environmental influences
Soothing effect
Preventive anti-pollution effects

2 **Humidity maintenance**
Epidermal hydration support for a balanced water content in skin

3 **Protection of dermal structures**
Protection of collagen and elastin – preserving skin‘s strength and elasticity
Protection from blue light-induced damage

4 **Skin refining**
Increased collagen network
Smoothing and wrinkle-reducing effects
Improved skin‘s aspect

SKIN CARE EMPOWERED BY NATURE

RonaCare® Luremin®
Technical details

Product no.	130204
INCI	Sorbitol, Dihydroxy Methylchromone
Appearance	Whitely-beige powder
Source	Plant and synthetic origin
Recommended use level	1 – 2 %, representing 0.05 - 0.1 % DHMC
Formulation aspects	**RonaCare® Luremin®** can be added directly to the formulation. In emulsions, it should be added as water dispersion after the emulsification step. Light stable, heat stable (> 6 months at 40 °C, >1h at 80 °C), stable towards oxidation via H_2O_2
Key active ingredient	Dihydroxymethylchromone (DHMC) ~ 5 %
Regulation & Labels	Vegan | Halal certified ISO 16128: 95 % natural origin content*

** The determination of the respective contents is based on our current knowledge and interpretation of the ISO 16128 Guideline. Therefore, product assessment may be adapted and a change of the respective determination may occur.*

RonaCare® Luremin®

SUGGESTED APPLICATIONS

- Daily skin care – Face & Body
- Age-defying products
- Dermocosmetic products
- Sun care
- Color cosmetics

Suitable formats: emulsions (o/w, w/o), gels, serums, ….

MADE TO REFINE

RONACARE® LUREMIN®

- Nature-identical, sustainable solution for self-care and well aging
- Tightening and refining
- Smoother, more supple skin
- Wrinkle-reducing effects
- Increased skin resistance to pollution and blue light stress

SKIN CARE EMPOWERED BY NATURE

RonaCare® Luremin®

FEATURES AT A GLANCE

- Sustainable & traceable production process
- Active compound of high purity
- Efficacy proof
- Labels: Halal certificate, Vegan
- Patent protected application

General

- Compliance with EFfCI GMP Standard for cosmetic ingredients
- Quality management ISO 9001:2015 & Energy management ISO 50001:2011
- Merck's Sustainability Report: more details here

Merck's 3 overall

SUSTAINABILITY GOALS

1. Dedicated to human progress
2. Creating sustainable value chains
3. Reducing our ecological footprint

Focus SDGs

(Sustainable Development Goals)

Sustainable aspects at supplier's side are considered

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03

RonaCare® Luremin®

EFFICACY TESTING

- Gene expression
- Skin cohesion & Skin defense
- Humidity maintenance
- Protection of dermal structures
- Skin refining

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RonaCare® Luremin® - Efficacy testing
Gene expression

Back to efficacy overview

Target	Test description	Results
Gene expression pattern in the epidermis	*In vitro*, human keratinocytes DHMC (0.04 mM) cDNA microarrays; Selective confirmation by qPCR	› Up-regulation of S100A8/A9, IGF1 › Strong up-regulation of involucrin › Down-regulation of syndecan-4, SOD1
Gene expression pattern in the dermis	*In vitro*, human fibroblasts DHMC (0.2 mM) cDNA microarrays	› Up-regulation of collagen III, VI › Up-regulation of fibronectin
Gene expression pattern in the dermis	*In vitro*, human fibroblasts RonaCare® Luremin® (0.1 %) RT-PCR	› Up-regulation of several collagens and extracellular matrix genes (elastin, laminin) › Down-regulation of hyaluronidase and collagenase

Gene expression
Effect of DHMC in human keratinocytes

Genes significantly modulated by DHMC:
Relative expression vs untreated control (%)
>200 %: up-regulation; <50 %: down-regulation

Gene	Rel. expression cDNA microarray	Rel. expression qPCR
S100A8	282 %	274 %
S100A9	265 %	218 %
IGF1	210 %	
Involucrin	201 %	356 %
Syndecan-4	34 %	
SOD1	44 %	

Test methods: Human keratinocytes (NEHK) were incubated for 24h in SFM medium in the presence of DHMC (40 µM).
First, gene expression profiling was performed using cDNA microarrays. In a second experiment, the effects on selective genes was evaluated using RT-qPCR and RNA isolated from new NHEK cultures

A **DNA microarray** (or DNA chip) is a collection of microscopic DNA spots attached to a solid surface. This technique is a powerful tool to determine the expression levels of large numbers of genes simultaneously.

DHMC, the active ingredient in **RonaCare® Luremin®**

- Up-regulates S100A8/A9, IGF1
- Strongly up-regulates **involucrin**
- Down-regulates syndecan-4, SOD1

in human keratinocytes

Gene expression
Effect of DHMC in human fibroblasts

Genes significantly modulated by DHMC:
Relative expression vs untreated control (%)
>200 %: up-regulation; <50 %: down-regulation

Gene	Rel. expression cDNA microarray
COL3A1	210 %
COL6A1	298 %
FN1	285 %

Test method: Human keratinocytes (NEHK) were incubated for 24h in SFM medium in the presence of DHMC (0.2 mM). Gene expression profiling was performed using cDNA microarrays.

Epstein, H. Euro Cosmetics (2017) 7-8, 26-32

A **DNA microarray** (or DNA chip) is a collection of microscopic DNA spots attached to a solid surface. This technique is a powerful tool to determine the expression levels of large numbers of genes simultaneously.

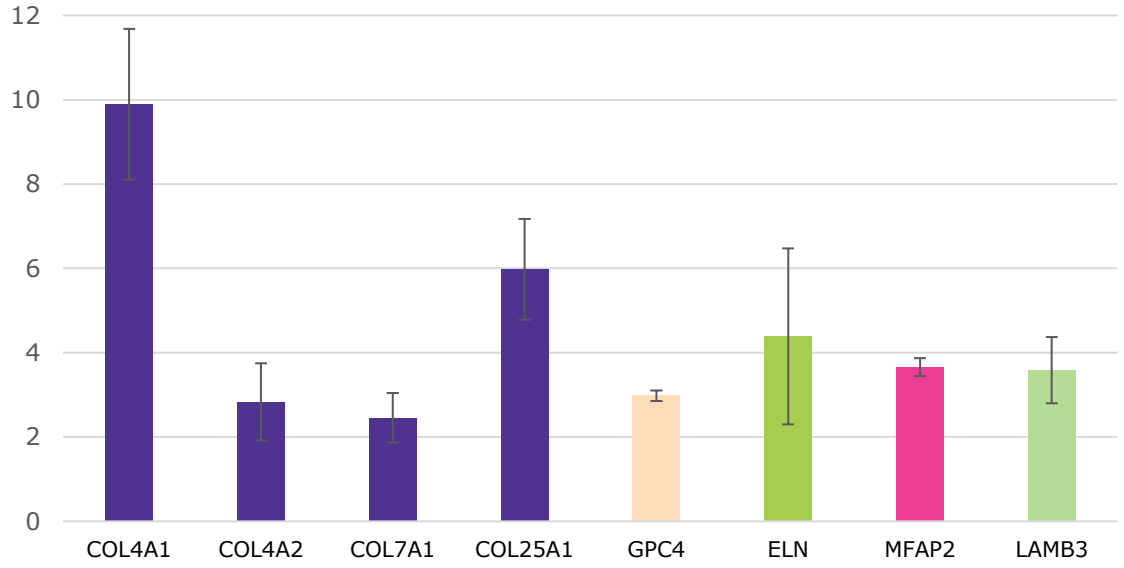
DHMC, the active ingredient in **RonaCare® Luremin®**

- Up-regulates collagens type III and VI
- Up-regulates **fibronectin**

in human fibroblasts

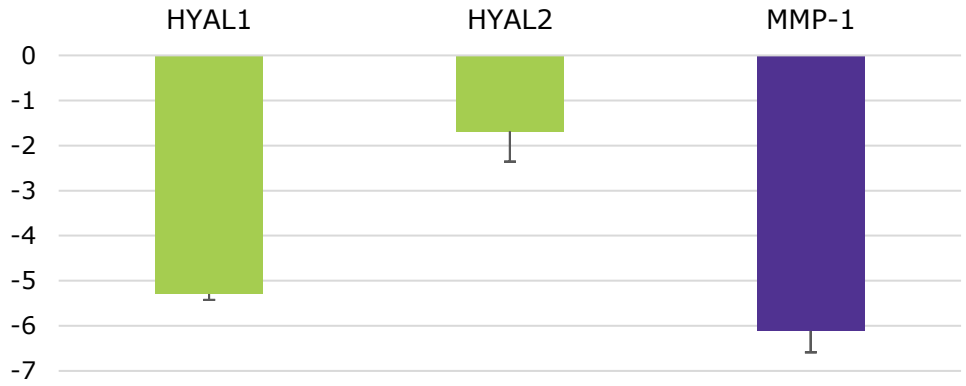
Gene expression
Effect of RonaCare® Luremin® in human fibroblasts

Significantly up-regulated genes
Fold change vs untreated control; > +2: up-regulation



Test method: The cells of skin fibroblast cell line WS-1 were cultivated at a seeding density of $1x10^6$ and treated with 0.1 % RonaCare® Luremin® for 48h (37 °C, 5 % CO_2). RNA was then isolated and used for gene expression analysis with the help of custom gene expression panels using the LC480 system (Roche, Germany)

Significantly down-regulated genes
Fold change vs untreated control; < -2: down-regulation



RonaCare® Luremin®

- Up-regulates several collagens and extracellular matrix genes: elastin, laminin,…
- Strongly down-regulates **hyaluronidase-1** and **MMP-1**

Glossary – Genes and function of their related proteins

Gene abbr.	Name	Function
S100A8/A9	Calgranulin A & B	Role in inflammation; Control wound healing by reorganizing the keratin cytoskeleton in the epidermis
IGF1	Insulin-like growth factor I	Polypeptide with high sequence similarity to insulin. The IGF axis has been shown to play roles in the promotion of cell proliferation and the inhibition of cell death (apoptosis)
IVL	Involucrin	Protein precursor of the epidermal cornified envelope. Its expression is initiated early in the epidermal differentiation process. Ultimately it becomes cross-linked to membrane proteins, helping in the formation of an intact skin barrier
SDC4	Syndecan 4	Cell-surface proteoglycan activating signaling pathways involved in inflammation, cell renewal and wound healing, as well as ECM and adhesion
SOD1	Superoxide dismutase 1	Antioxidant enzyme protecting the cell from reactive oxygen species toxicity
COL4A1/A2	Collagen type IV alpha-1/2	Essential part of the lamina densa component of the epidermal basement membrane, which anchors it to the dermis. Reduced with age.
COL6A3	Collagen type VI alpha-3	Found immediately below the dermal-epidermal junction. Associates with type I and type III collagen and is involved in cell–cell and cell–matrix communication, adhesion, and organization.
COL7A1	Collagen type VII alpha-1	The major structural fibril component of the achorage fibrils in the dermal-epidermal junction. Reduced with age.
COL25A1	Collagen type XXV alpha-1	Transmembrane collagen expressed in dermal fibroblasts closely related to collagen XVII, both of which play an important role in dermal-epidermal junction anchorage. Role continues to be elucidated.
FN1	Fibronectin	Glycoprotein functioning both as a regulator of cellular processes and an important scaffolding protein to maintain and direct tissue organization and ECM composition
GPC4	Glypican 4	Heparan sulfate proteoglycan involved in numerous cell functions, including regulating cell proliferation and cell survival
ELN	Elastin	One of the main constituents of the elastic fibers in the papillary and reticular dermis, having an important function in providing elasticity to the skin
MFAP2	Microfibril associated protein 2	Together with fibrillin-1, it is a component of extracellular microfibrils. Involved in matrix organisation and part of the skin elastic fiber network.
LAMB3	Laminin subunit beta 3	Laminins are glycoproteins that regulate cell growth, motility and adhesion. They are also involved in the formation and organization of basement membranes

Back to efficacy overview

GENE EXPRESSION ANALYSIS

RONACARE® LUREMIN®
Dihydroxymethylchromone

DERMIS

Up-regulation of several structural genes of the extracellular matrix: collagens, elastin, fibronectin, laminin

Down-regulation of degrading enzymes HYAL1 (hyaluronidase) and MMP-1 (collagenase)

EPIDERMIS

Up-regulation of S100A8/A9 and IGF1, enabling an early response to stress; involucrin, promoting skin cohesion

Down-regulation of syndecan-4, SOD1 as result of reduced inflammatory onset

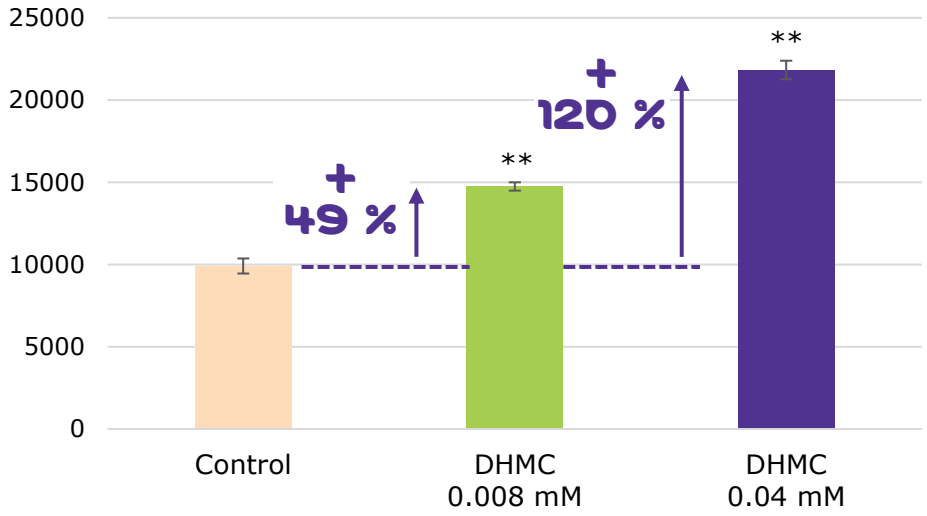
RonaCare® Luremin® - Efficacy testing
Skin cohesion & Skin defense

Back to efficacy overview

Target	Test description	Results
Skin cohesion	*In vitro*, human keratinocytes DHMC (0.008 mM, 0.04 mM) Effect on keratinocyte differentiation using a transglutaminase activity assay	› Significant increase of TGM1 activity – up to +120 % vs control
Skin defense	*In vitro*, human keratinocytes DHMC (0.04 mM, 0.2 mM) Soothing effect under irritation stress	› Dose-dependent decrease of PGE2 release after induction of an irritation stress – up to 98 % inhibition vs control
Skin defense	*Ex vivo*, human skin explants DHMC (0.1 % in PG/H_2O 70:30 solution) Impact of pollution exposure on markers of oxidative stress involved in cell protection (Nrf2) and detox (LC3B)	› Significant reduction of pollutant-induced Nrf2 activation by 32 % vs placebo › Significant reduction of pollutant-induced LC3B expression by 53 % vs placebo

Skin cohesion
Skin barrier support

TGM1 activity in keratinocytes
(cpm/well, n=3)



Test method: Human keratinocytes (NEHK) were incubated for 96h in SFM medium supplemented with Ca^{2+} (1.5 mM) and in the presence of DHMC. The enzyme TGM1 was extracted from cell membranes and its activity was assayed using liquid scintillation. Statistics: ANOVA, using multiple Dunnett's comparison test; ** $p<0.01$ vs control (+Ca^{2+})

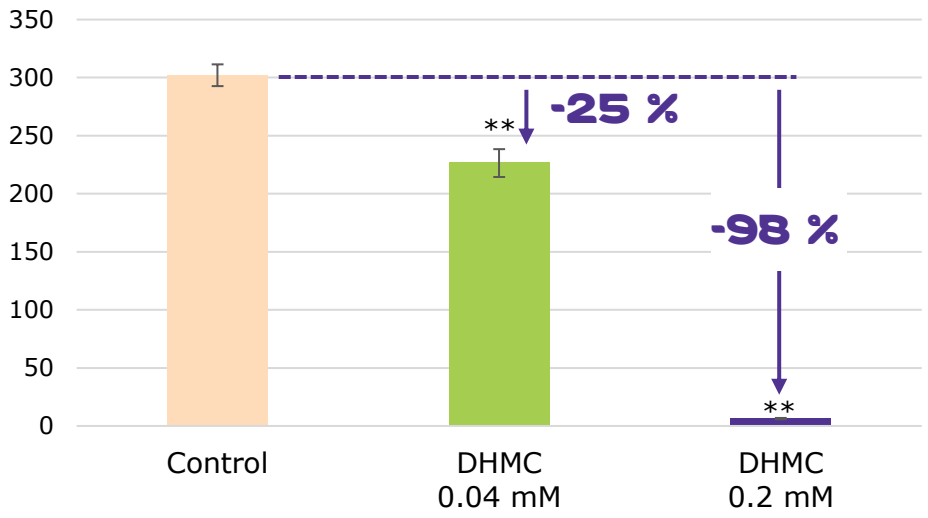
TGM1 (transglutaminase 1) is a crucial enzyme involved in the keratinization process through crosslinking of cornified envelope proteins incl. involucrin, loricrin, and SPRs (small proline-rich proteins). Transglutaminases play an essential role in maintaining the barrier function of the skin. TGM1 activity is significantly increased in Ca^{2+} containing media.

DHMC, the active ingredient in **RonaCare® Luremin®**

- Significantly **increases TGM1** activity in keratinocytes – up to 120 % stimulation
- Together with **involucrin up-regulation**, this contributes to support an efficient skin **barrier function**

Skin defense
Soothing effect

PGE2 release by keratinocytes
(ng/ml, n=3)



Test method: Human keratinocytes (NCTC) were treated with DHMC or a positive reference (indomethacin, 1µM). PMA (phorbol myristate acetate, 0.1 µg/ml) was added to induce an inflammatory reaction. The cells were incubated for 24h and the release of inflammation marker PGE2 was measured by ELISA. Statistics: ** $p<0.01$ vs control (+PMA)

PGE2 (prostaglandin E2) is a lipid signaling mediator of the arachidonic pathway known to play a crucial role in inflammation. It is produced by both epidermal keratinocytes and dermal fibroblasts and contributes to homeostatic processes and inflammatory responses associated with injury, allergy and other acute or chronic conditions.

DHMC, the active ingredient in **RonaCare® Luremin®**

- Strongly **reduces the stress-induced release of PGE2** by keratinocytes – up to 98 % inhibition
- Helping to **suppress skin irritation**

Skin defense
Anti-pollution test

Objective

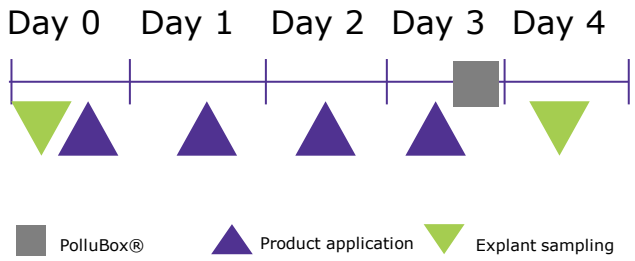
Evaluate the the anti-pollution activity of **DHMC**, the active in **RonaCare® Luremin®** after application of a pollutant mix on human skin explants

Readouts: Immunostaining + semi-quantification of:

I. **Nrf2 activation**
II. **LC3B expression**

Test products

- **DHMC** (0.1 % in PG/H_2O 70:30)
- Placebo: PG/H_2O 70:30
- **Product application:**



Explants

- **Skin explants** from an abdoplasty of a 55 yr old Caucasian woman
- **Explants** kept in survival in BEM culture medium at 37 °C in a humid, 5 % - CO_2 atmosphere

Pollutant mix – PolluBox®

- **Heavy metals (HM) mixture** containing Al, As, B, Ba, Be, Ca, Cd, Cr, Cu, Fe, Hg K, Li, Mg, Mn, Na, Ni, P, Pb, Sa, Sc, Se, Sr, Te, Ti, Y, Zn
- **Hydrocarbons** (benzene, xylene, toluene) **+ Diesel particles** (0.001 %) **PM2.5**

Skin defense

Preventive anti-pollution effect – Response to oxidative stress

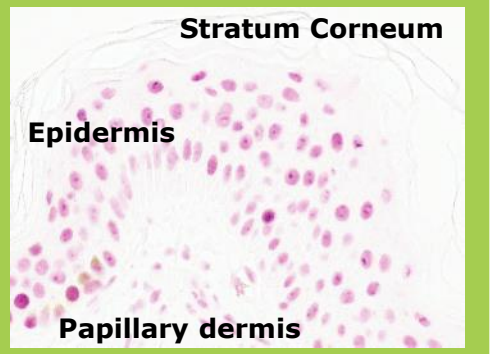
- 32 %

Nrf2 activation

- **Nrf2** is a key transcription factor in the **cellular response to oxidative stress**
- Nrf2 is activated under oxidative stress and translocates from the cytoplasm to the nucleus
- Once in the nucleus, it induces the biosynthesis of antioxidative enzymes
- **A decrease of Nrf2** activation is associated with a **preventive anti-pollution** effect

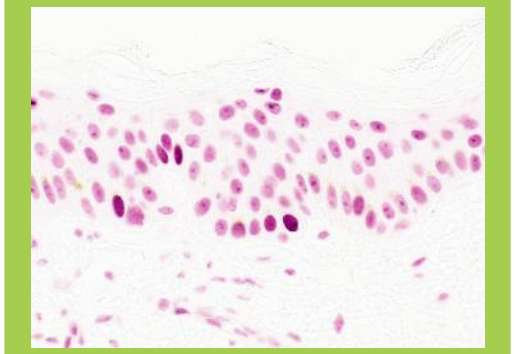
% surface occupied by Nrf2 in the epidermis of skin explants on Day 4
w/ or w/o treatment with a pollutant mix (PM 2.5 + HM) for 24h on Day 3
(Topical application of DHMC 0.1 % on skin explants over 3 days)

Placebo control



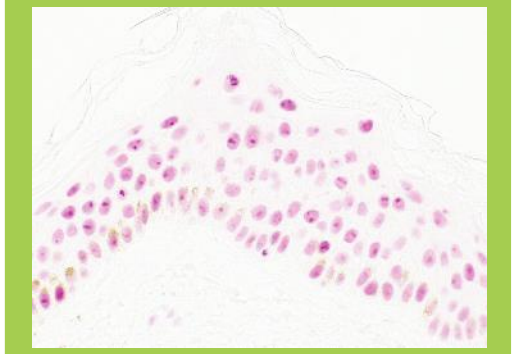
- **10.2 %** of the surface is occupied by Nrf2 in the epidermis on Day 4

Placebo + pollutants



- **17.5 %** of surface occupied by Nrf2 after pollution stress
- Significant increase of Nrf2 activation:
  vs. placebo control + 72 %**

DHMC + pollutants



- **11.9 %** of the surface is occupied by Nrf2 after pollution stress
- **Significant decrease of Nrf2 activation:**
  vs. polluted control - 32 %**

* $p<0.05$; ** $p<0.01$ (Student t-test)

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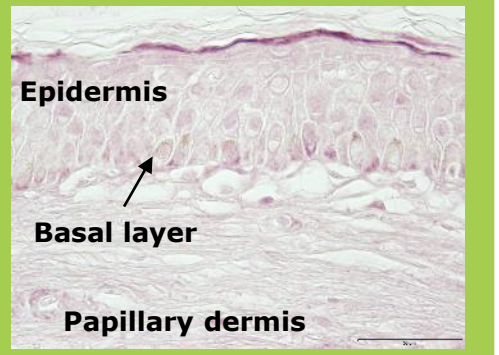
Skin defense
Preventive anti-pollution effect – Effect on autophagy

- 53 %
LC3B expression

- **LC3** is a protein involved in **autophagy**, a process of cell material recycling leading to degradation of e.g., damaged proteins, oxidized lipids etc.
- **LC3B** is the biological (lipidic) active form of LC3
- In skin, the autophagy process is stimulated by oxidative stress contributing to the cell detoxifying process
- **A decrease of LC3B** expression is associated with a **preventive anti-pollution** effect

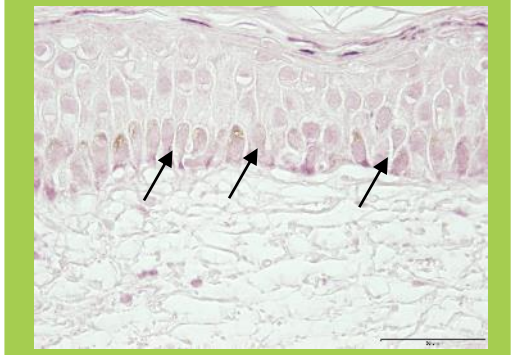
% surface occupied by LC3B in the basal layer of skin explants on Day 4
w/ or w/o treatment with a pollutant mix (PM 2.5 + HM) for 24h on Day 3
(Topical application of DHMC 0.1 % on skin explants over 3 days)

Placebo control



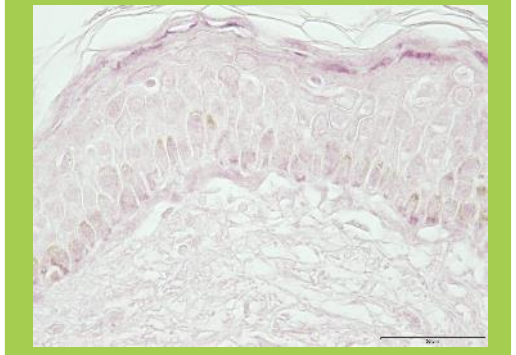
- **6.9 %** of the surface is occupied by LC3B in the basal layer on Day 4

Placebo + pollutants



- **10 %** of surface occupied by LC3B in response to the pollution stress
- Significant increase of LC3B expression:
  vs. placebo control **+ 45 %**

DHMC + pollutants



- **4.7 %** of the surface is occupied by LC3B after pollution stress
- **Significant decrease of LC3B expression:**
  vs. polluted placebo **- 53 %***

* $p<0.05$ (Student t-test)

Back to efficacy overview

INCREASED SKIN RESISTANCE TO ENVIRONMENTAL INFLUENCES

RONACARE® LUREMIN®
Dihydroxymethylchromone

SKIN COHESION

Increased activity of transglutaminase plus up-regulation of involucrin positively influence the **keratinization** process – for a strengthened **barrier function**

SKIN DEFENSE

Soothing effect – demonstrated by a decrease of stress-induced prostanglandin E2 formation

Preventive anti-pollution effects, as shown by the reduction of Nrf2 and LC3B – 2 markers of oxidative stress induced by exposure to pollutants

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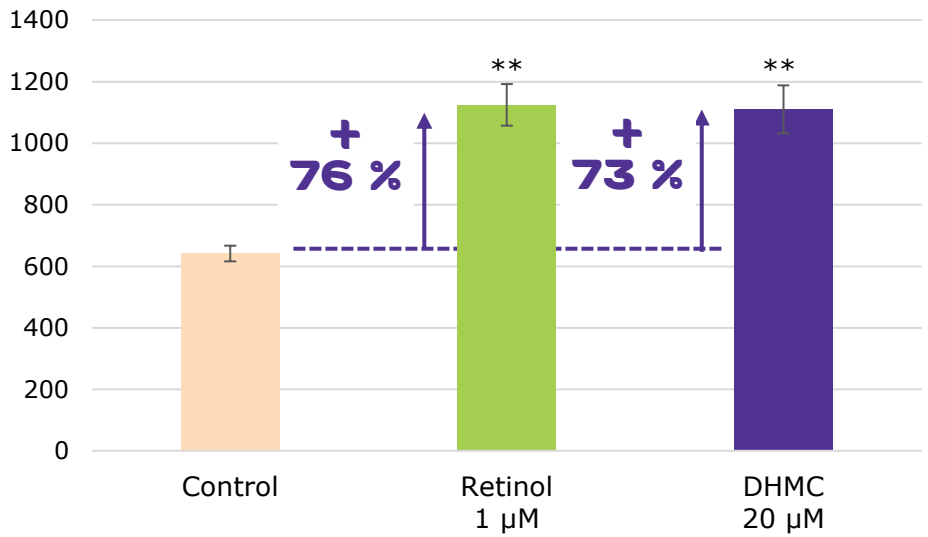
RonaCare® Luremin® - Efficacy testing
Humidity maintenance

Back to efficacy overview

Target	Test description	Results
Skin hydration support	*In vitro*, human keratinocytes DHMC (20 µM) Effect on hyaluronic acid synthesis	› Stimulates HA synthesis by 73 % vs control
Moisture retention	*In vitro*, biochemical assay DHMC (3-25 mM) Inhibitory effect on hyaluronidase	› Dose-dependent inhibition of hyaluronidase

Humidity maintenance
Stimulation of hyaluronic acid

HA synthesis by keratinocytes
(ng/ml, n=3)



Test method: Human keratinocytes (NEHK) were incubated for 72h in SFM medium in the presence of DHMC or retinol (positive control). The concentration of hyaluronic acid in the supernatants was evaluated by ELISA. Statistics: ANOVA, using multiple Dunnett's comparison test; ** $p<0.01$ vs control

HA (hyaluronic acid) is a polysaccharide playing an important role in tissue hydration and water transport due to its huge water-binding capacity. The skin content of HA decreases with aging, especially epidermal HA which can almost entirely disappear in aged skin. This contributes to moisture loss; the skin becomes thinner and less supple.

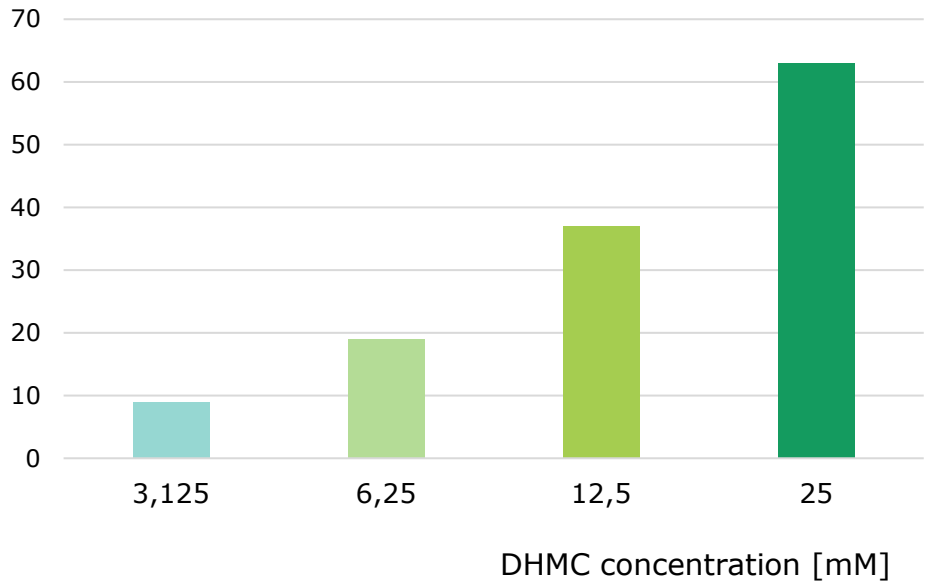
DHMC, the active ingredient in **RonaCare® Luremin®**

- Significantly stimulates the **synthesis of hyaluronic acid** by keratinocytes
- Helping to improve the **hydration status** of skin's upper layers

Carola, C. et al. SOFW Journal (2010) 136, 4:2-10

Humidity maintenance
Protection of hyaluronic acid

Hyaluronidase inhibition [%] (n=3)



Test method: DHMC at different concentrations and hyaluronidase (1 mg/ml) were pre-incubated in appropriate buffers. Hyaluronic acid (HA, 1.2 mg/ml) was added and the mix was incubated for 1h. Residual HA was precipitated with BSA and photometrically quantified at 540 nm.

Hyaluronidase is an enzyme that degrades hyaluronic acid. The inhibition of this enzyme can therefore help limit the decrease of hyaluronic acid content in skin.

DHMC, the active ingredient in **RonaCare® Luremin®**

- Significantly **inhibits the hyaluronidase** activity with an IC_{50} of approx. 20 mM
- This corroborates the down-regulating effect of **RonaCare® Luremin®** on HYAL1 gene expression
- This is helpful to **preserve** the levels of hyaluronic acid in skin

Carola, C. et al. SOFW Journal (2010) 136, 4:2-10

Back to efficacy overview

EPIDERMAL HYDRATION SUPPORT

RONACARE® LUREMIN®
Dihydroxymethylchromone

SKIN MOISTURE

Stimulation of hyaluronic acid synthesis – to support an efficient **water content** in skin

HUMIDITY MAINTENANCE

Protection of hyaluronic acid due to the inhibition of HA-degrading enzyme hyaluronidase – to **secure skin's moisture**

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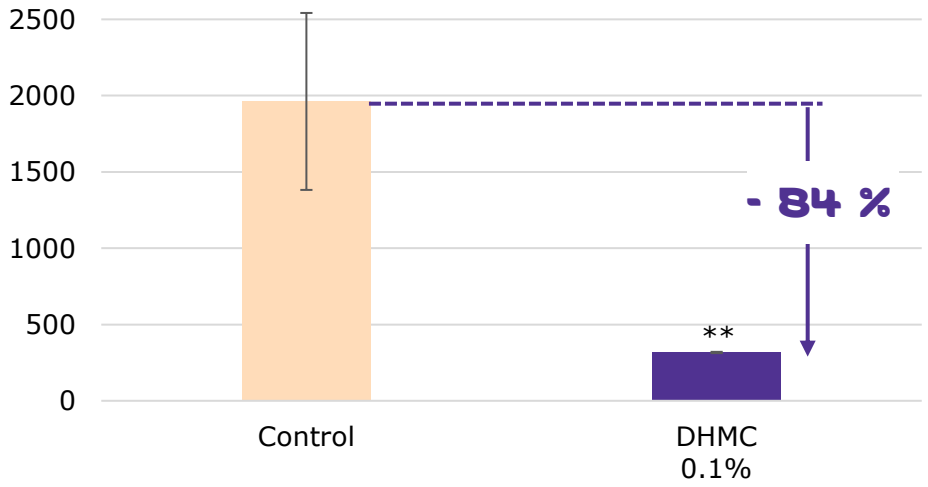
RonaCare® Luremin® - Efficacy testing
Protection of dermal structures

Back to efficacy overview

Target	Test description	Results
Protection of elastin	*In vitro*, biochemical assay DHMC (0.1 %) Inhibitory effect on elastase	› Significant elastase inhibition by 84 % vs control
Protection of collagen	*In vitro*, human fibroblasts DHMC (0.004 %) Inhibitory effect on collagenase	› Significant decrease of MMP-1 release by 40 % vs control
Photoprotection	*Ex vivo*, human skin explants DHMC (0.1 % in PG/H_2O 70:30 solution) Impact of blue light irradiation on MMP-1 expression	› Significant reduction of blue light-induced MMP-1 expression in the dermis by 41 % vs placebo

Protection of dermal structures
Protection of elastin

Elastase activity
(Fluorescence intensity, n=3)



Test method: DHMC (0.1 %) and elastase from human leucocyte (100 mU/ml) were pre-incubated in Tris buffer for 10 min. on ice. DQ-elastin (100 µg/ml) was added and the mix was incubated for 2h. After centrifugation, the filtrates were collected in microtubes containing the elastase inhibitor AAPV (0.1 mM), and immediately placed on ice. Each sample was measured at λex 485 nm, λem 538 nm. Statistics: ANOVA, using multiple Dunnett's comparison test ** $p<0.01$ vs control

Elastase is an enzyme that degrades elastin, one of the main constituents of the elastic fibers in the papillary and reticular dermis, having an important function in providing elasticity to the skin. The inhibition of elastase can therefore help limit the decrease of elastin content in the extracellular matrix.

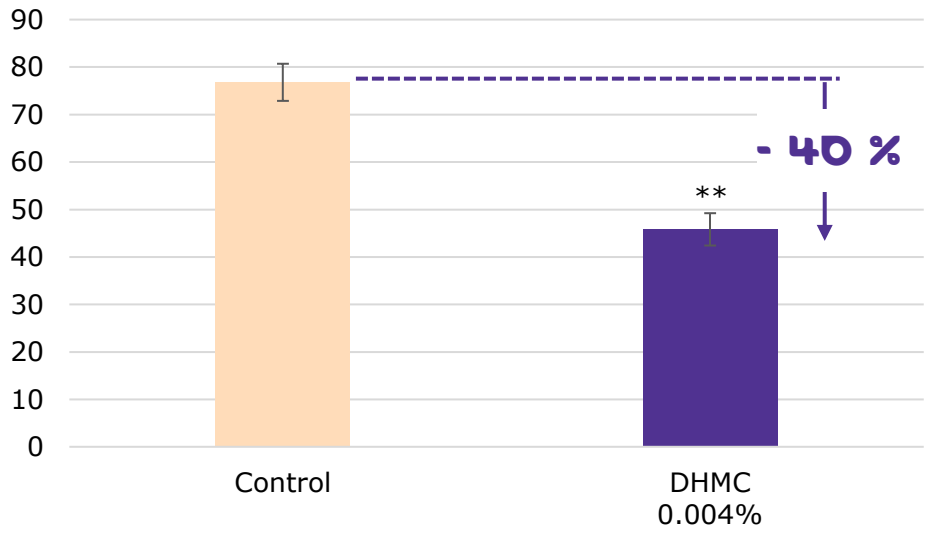
DHMC, the active ingredient in **RonaCare® Luremin®**

- Strongly **inhibits the elastase** activity – 84 % inhibition
- This is helpful to **slow down** the degradation process of elastin in aging skin

Carola, C. et al. SOFW Journal (2010) 136, 4:2-10

Protection of dermal structures
Protection of collagen

MMP-1 release from fibroblasts
(ng/ml, n=3)



Test method: Human fibroblasts (NHDF) were treated with DHMC or a positive reference (dexamethasone, 0.1µM). The cells were incubated for 24h and the MMP-1 content in the supernatants was measured by ELISA. Statistics: ANOVA, using multiple Dunnett's comparison test;
** $p<0.01$ vs control

MMP-1 (matrix metalloproteinase-1) is a matrix-degrading enzyme that breaks down the interstitial collagens, types I, II, and III. With age, the amount of collagen in the skin tends to decline due to lower levels of collagen synthesized by aged fibroblasts but also because the production of matrix metalloproteinases (MMPs) increases.

DHMC, the active ingredient in
RonaCare® Luremin®

- Significantly **reduces the basal release of MMP-1** from fibroblasts by 40 %
- This correlates with the downregulating effect of **RonaCare® Luremin®** on MMP-1 gene expression
- Helping to **limit collagen degradation**

Carola, C. et al. SOFW Journal (2010) 136, 4:2-10

Protection of dermal structures
Photoprotection – Blue light test

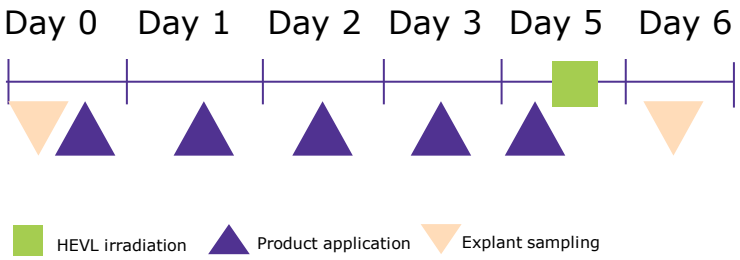
Objective

Evaluate the photo-protective activity of **DHMC**, the active in **RonaCare® Luremin®** against acute HEVL irradiation on human skin explants

Readout:
Immunostaining + semi-quantification of **MMP-1 expression**

Test products

- **DHMC** (0.1 % in PG/H_2O 70:30)
- Placebo: PG/H_2O 70:30
- **Product application:**

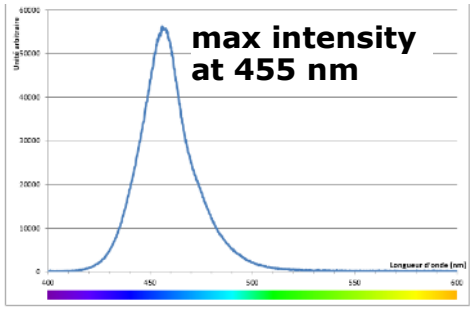


Explants

- **Skin explants** from a thigh plasty of a 62 yr old Caucasian woman
- **Explants** kept in survival in BEM culture medium at 37 °C in a humid, 5 % - CO_2 atmosphere

Irradiation – SolarBox®

- Dose: 63.75 J/cm² for 3h



Protection of dermal structures
ECM protection from Blue light-induced damage

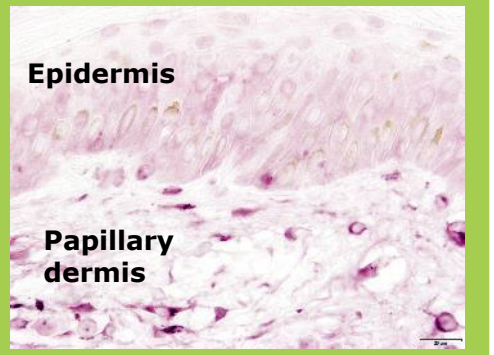
- 41 %

MMP-1 expression in the dermis

- **MMP-1 (Matrix metalloproteinase-1)** belongs to a family of peptidase enzymes responsible for the degradation of extracellular matrix components
- **MMP-1** also known as **collagenase,** can be induced by e.g., sunlight exposure, therefore leading to premature skin aging

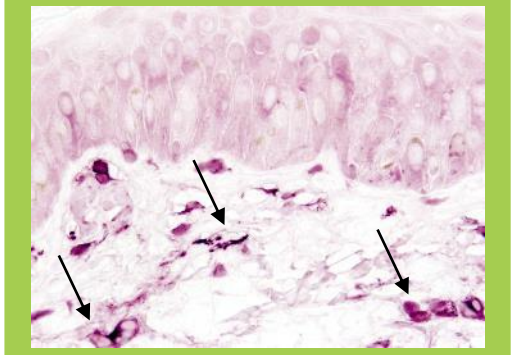
% surface occupied by MMP-1 in the dermis of skin explants on Day 6
w/ or w/o single dose HEVL irradiation (63.75 J/cm² for 3h) on Day 5
(Topical application of DHMC 0.1 % on skin explants over 5 days)

Placebo control



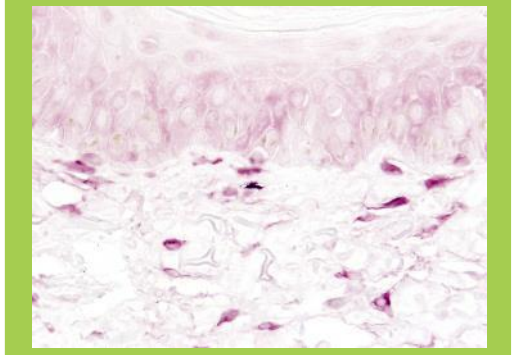
- **11.3 %** of the surface is occupied by MMP-1 in the papillary dermis on Day 6

Placebo + HEVL



- **13 %** of the surface is occupied by MMP-1 after HEVL irradiation
- Significant increase of MMP-1 expression:
- **vs. placebo control** **+ 15 %**

DHMC + HEVL



- **7.6 %** of the surface is occupied by MMP-1 after HEVL irradiation
- **Significant decrease of MMP-1 expression:**
- **vs. polluted placebo** **- 41 %****

** $p<0.01$ (Student t-test)

Back to efficacy overview

PROTECTION OF DERMAL STRUCTURES

RONACARE® LUREMIN®
Dihydroxymethylchromone

COLLAGEN

Protection of collagen due to the inhibition of collagen-degrading enzyme collagenase – to **maintain skin's strength**

Photoprotective effect, as shown by the inhibition of blue-light induced MMP-1 in the dermis

ELASTIN

Inhibition of elastin-degrading enzyme elastase – to **preserve skin's elasticity**

RonaCare® Luremin® - Efficacy testing
Skin Refining

Back to efficacy overview

Target	Test description	Results
Collagen synthesis	*Ex vivo*, human skin explants Treatment with RonaCare® Luremin® (2 % in o/w emulsion) for 8 days Immunostaining of total collagen	› Stimulation of collagen synthesis by 7 %; Significant increase vs placebo
Skin smoothing	*In vivo* study on 40 volunteers Treatment with DHMC (0.1 % in o/w emulsion) for 28 days Evaluation of cutaneous relief parameters using 3D Primos® Subjective evaluation of treatment efficacy	› Significant smoothing and anti-wrinkle effects: Reduction of average roughness by 12 % (Ra); Reduction of max relief amplitude by 8 % (Rt); Reduction of average relief by 11 % (Rz) › No significant effect of the placebo › Higher improvement of volunteers' skin's state and aspect compared to placebo treatment
Skin smoothing, comparative efficacy vs. retinol	*In vivo* study on 44 volunteers Treatment with RonaCare® Luremin® (2 % in o/w emulsion) for 28 days; Evaluation of anti-wrinkle effect, skin's biomechanical properties, effects on the cutaneous barrier, subjective evaluation of treatment efficacy	› Significant smoothing and anti-wrinkle effects, significant increase in skin firmness and elasticity, significant support of skin barrier › Mostly no significant effect of the comparative product containing retinol › Higher efiicacy perception compared to placebo and retinol-containing products

Skin refining
Collagen network

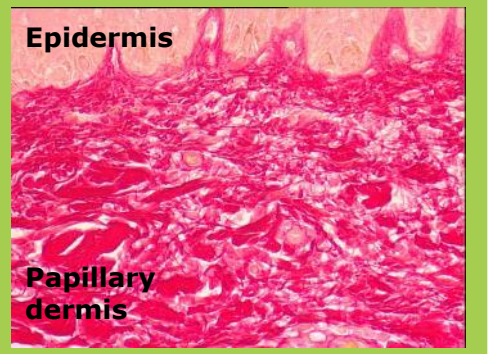
+ 7 %
Collagen induction in the dermis

Test method

- Skin explants from cosmetic surgeries of a 46 yr old woman
- Topical application of **RonaCare® Luremin®** (2 % in o/w emulsion) or placebo o/w for 8 days
- Day 8: sampling of the explants, control of cellular viability and **immunostaining of total collagen**, followed by image analysis

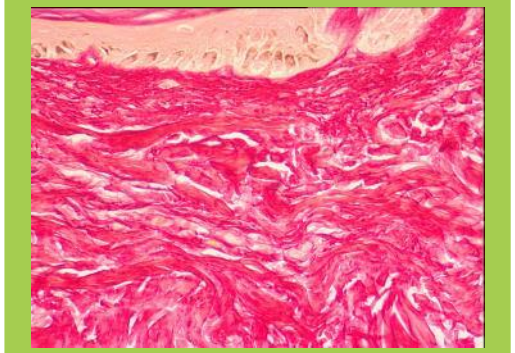
% surface occupied by collagen in the dermis (papillary and upper reticular dermis) on Day 8

Untreated control



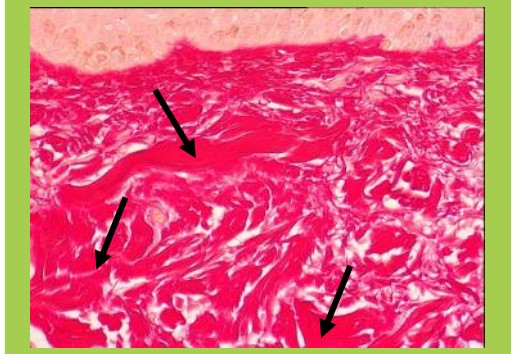
- **86.8 %** of the surface is occupied by collagen
- Collagen fibers are moderately thick forming a quite dense network

Placebo



- **85.1 %** of surface occupied by collagen
- Collagen fibers are moderately thick forming a quite dense network

RonaCare® Luremin®



- **91.5 %** of surface occupied by collagen
- Collagen fibers are thick forming a dense network; clear density increase

vs. Placebo

+ 7 %***

*** $p<0.001$ (Student t-test)

Carola, C. et al. SOFW Journal (2010) 136, 4:2-10

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Skin refining – DHMC
Anti-wrinkle study – Test design

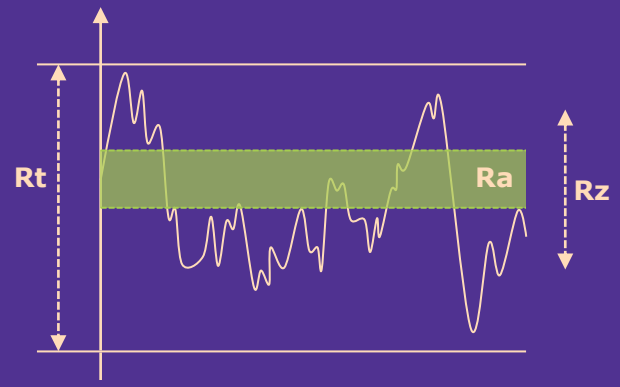
- **Study Type:**
  Double blind, placebo-controlled inter-individual study;
  Before / After treatment
- **Assessments:**
  Quantification of the cutaneous relief on crow's feet area using 3D Primos®; Illustrative photos; Subjective evaluation questionnaire on product efficacy
- **Volunteers:**
  2 groups of 20 women aged 44-62 yrs, mean age 56 ±1 yr with wrinkles around the eyes (crow's feet)
- **Test products:**
  Base o/w emulsion (Placebo); Base emulsion with DHMC 0.1 % (representing RonaCare® Luremin® 2 %)
- **Application:**
  2x daily application on whole face for 28 days

Evaluation of cutaneous relief with 3D-Primos®*

Ra: Average roughness

Rt: Maximum relief amplitude

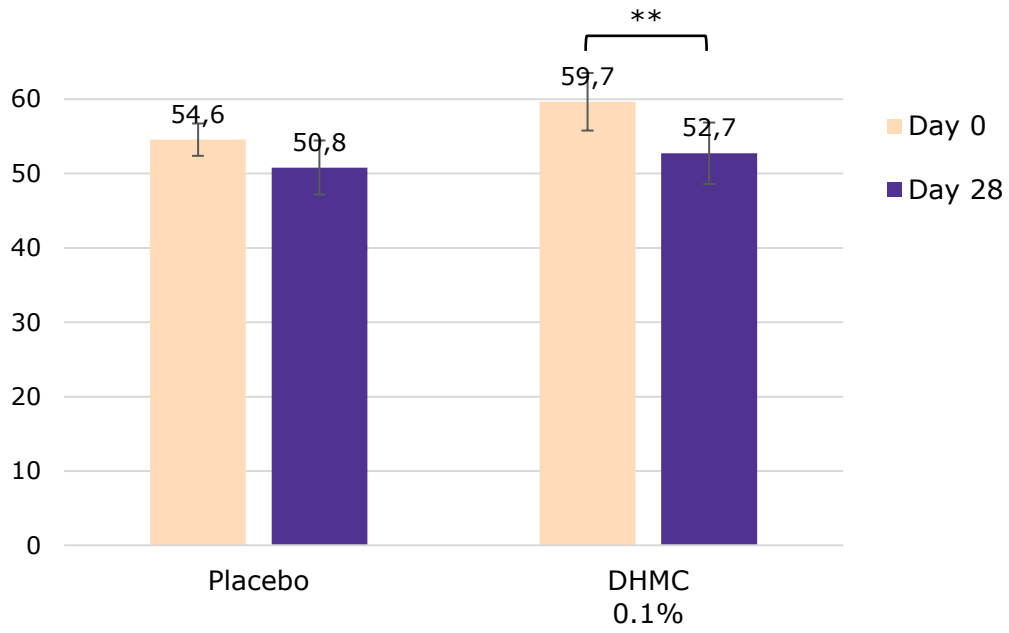
Rz: Average relief



* *Phaseshift Rapid In vivo Measurement Of Skin*

Skin refining – DHMC
Skin smoothing

Ra: Average roughness (µm, n=20)



-12 %
average roughness
with DHMC 0.1 %

** $p<0.01$ (Student t-test)

Ra: Average roughness
Represents the arithmetic mean of the absolute values of the heights of the profile length. A decrease in Ra characterizes a **smoothing effect.**

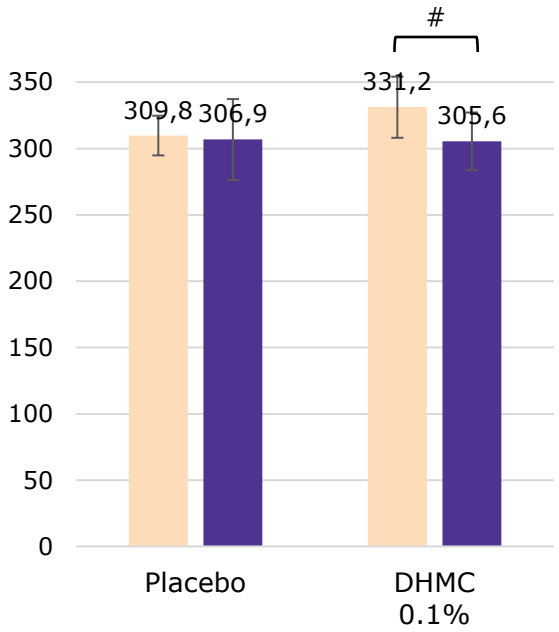
DHMC, the active ingredient in **RonaCare® Luremin®**

- Significantly **decreases** the **average roughness** around the eyes by **12 %**
- A **smoothing effect** could be observed in **80 %** of the volunteers
- The placebo product had no significant effect

Carola, C. et al. SOFW Journal (2010) 136, 4:2-10

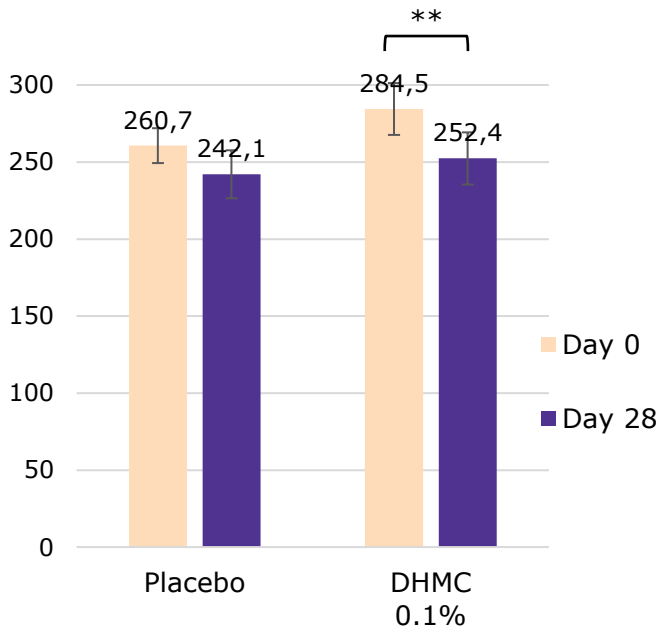
Skin refining – DHMC
Wrinkle reduction

Rt: Maximum relief amplitude (µm, n=20)



-8 %
max relief amplitude
with DHMC 0.1 %

Rz: Average relief (µm, n=20)



-11 %
average relief
with DHMC 0.1 %

# $p<0.1$; ** $p<0.01$ (Student t-test)

Carola, C. et al. SOFW Journal (2010) 136, 4:2-10

Rt: Maximum relief amplitude
Height difference from the highest peak, to the deepest valley within the total measurement section.

Rz: Average relief
Average of 5 single roughness depths (difference from the highest profile peak, to the deepest profile valley within a single measurement section).

A decrease in Rt and Rz parameters characterizes an **anti-wrinkle effect.**

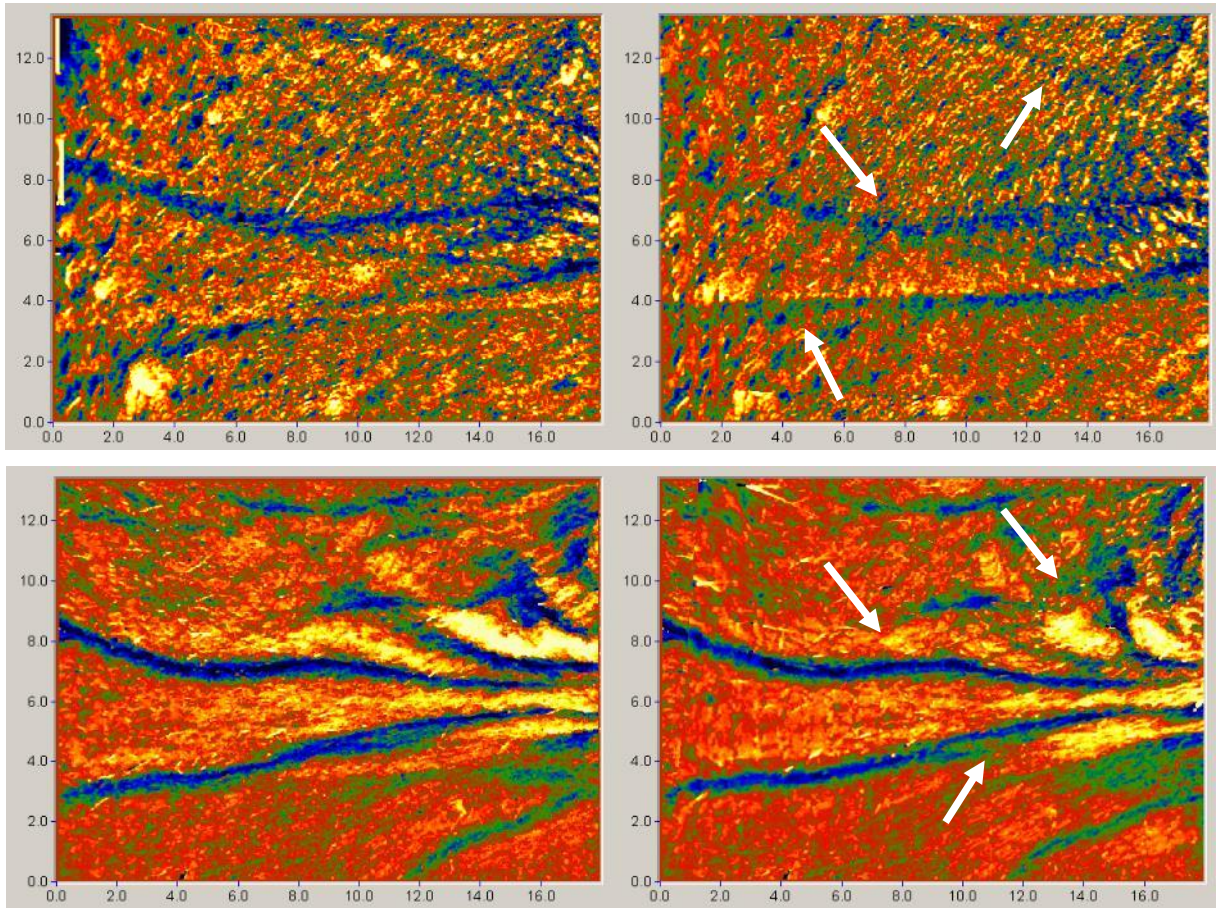
DHMC, the active ingredient in **RonaCare® Luremin®**

- Shows **significant anti-wrinkle** effects by decreasing the cutaneous relief parameters Rt and Rz by **8 % and 11 %** respectively
- Positive effects could be measured in **80-85 %** of the volunteers
- The placebo had no significant impact

Skin refining – DHMC
Visible skin smoothing effect

before treatment (D0)

after treatment with DHMC 0.1 % (D28)



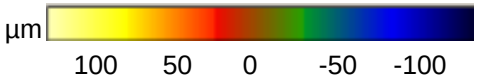
Exemplary pictures and 3D-Primos® values

44 yr old woman, D28-D0

Ra: -5.1 µm (-14.5 %)
Rt: -52.8 µm (-22.8 %)
Rz: -33.0 µm (-15.9 %)

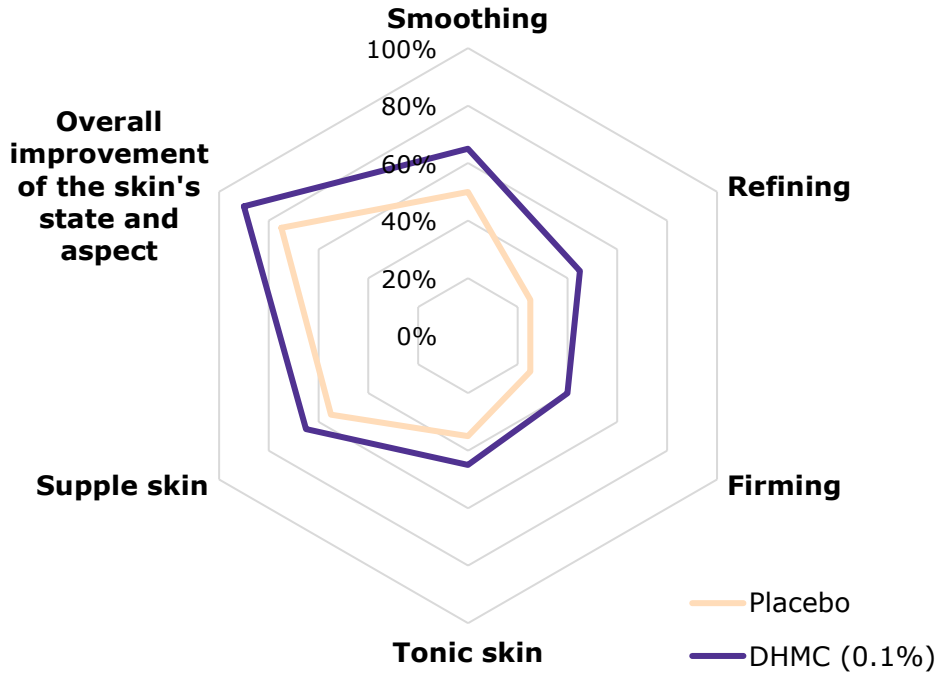
51 yr old woman, D28-D0

Ra: -6.8 µm (-10.7 %)
Rt: -79.9 µm (-19.4 %)
Rz: -38.6 µm (-11.7 %)



DHMC, the active ingredient in **RonaCare® Luremin® visibly reduces** fine lines and wrinkles

Skin refining – DHMC
Perceived skin improvements



Test method: After completion of the study, the volunteers were asked to evaluate if the test products were able to improve specific skin parameters. The graph shows the percentage of positive answers for the respective criteria

Subjective evaluation by the test subjects

The test product containing **DHMC,** the active ingredient in **RonaCare® Luremin®**, was found to be **more effective** in many aspects:

- **90 %** of the volunteers have noticed an **improvement of their skin`s state** and aspect (compared to 75 % of placebo users)
- **65 %** stated to have a **smoother** (50 % in the placebo group), **more supple skin**
- **45 %** found their skin **refined** (only 25 % of placebo users) and **more tonic**

Skin refining – RonaCare® Luremin®
Comparative efficacy vs. retinol – Test design

- **Study Type:**
  Double blind, placebo-controlled inter-individual study;
  Before / After treatment
- **Assessments:**
  Anti-wrinkle effects on crow's feet; Effect on skin's biomechanical properties; Effect on the cutaneous barrier; Subjective evaluation questionnaire; Illustrative photos and 2D & 3D pictures
- **Volunteers:**
  34 women and 10 men aged 45-65 yrs, mean age 55 ±1 yr with wrinkles around the eyes (crow's feet), dry to very dry skin on face, loose skin, cutaneous imperfections (blotches, diffuse redness)
- **Application:**
  2x daily application on hemi-faces for 28 days:
  1 group tested RonaCare® Luremin® vs Placebo;
  1 group tested RonaCare® Luremin® vs Comparator

PRODUCTS

Placebo: Base o/w emulsion

tested by 22 subjects

Test product: Base emulsion with **2 % RonaCare® Luremin®** (representing 0.1% DHMC)

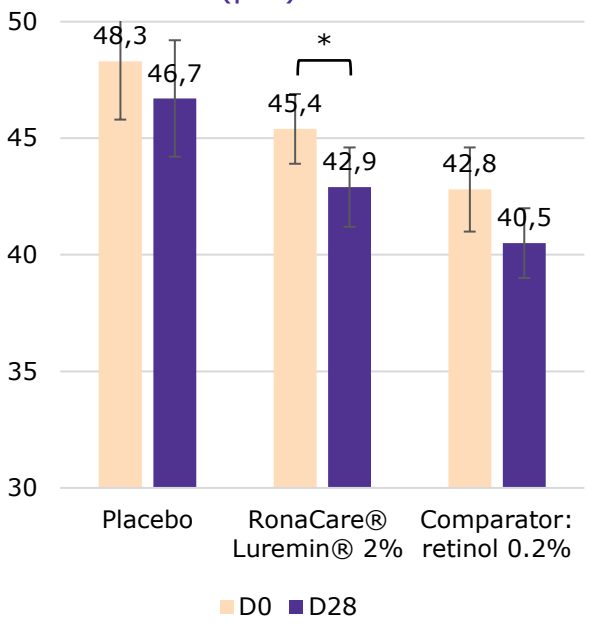
tested by 44 subjects

Comparator: Market product (o/w) with **0.2 % retinol**

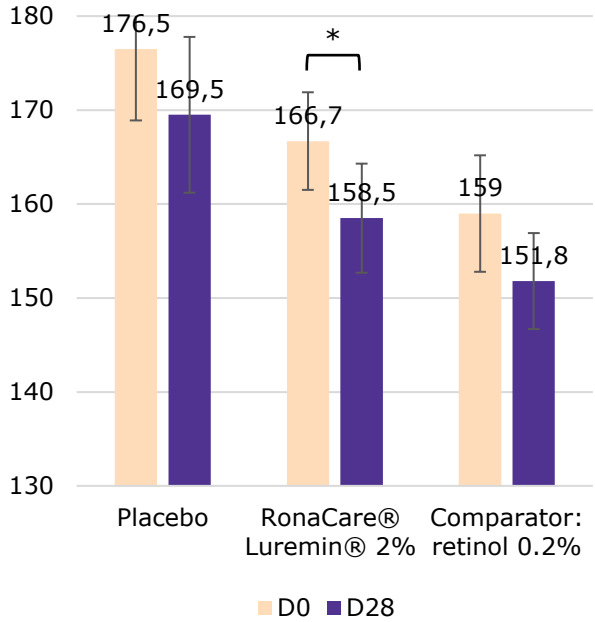
tested by 21 subjects

Skin refining – RonaCare® Luremin®
Skin smoothing & Wrinkle reduction

Ra: Average roughness (µm)



Rz: Average relief (µm)



-5 %

average roughness & average relief

with RonaCare® Luremin® 2%

* $p<0.05$ (ANOVA)

Ra: Average roughness

Arithmetic mean of the absolute values of the heights of the profile length. A decrease in Ra characterizes a **smoothing effect.**

Rz: Average relief

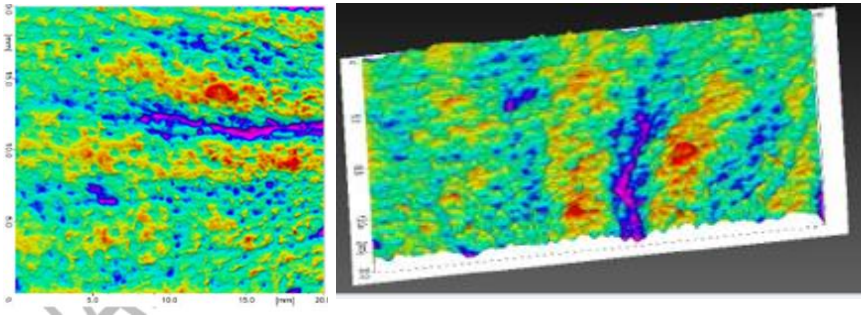
Average of 5 single roughness depths (difference from the highest profile peak, to the deepest profile valley within a single measurement section).A decrease in Rz characterizes an **anti-wrinkle effect.**

RonaCare® Luremin®

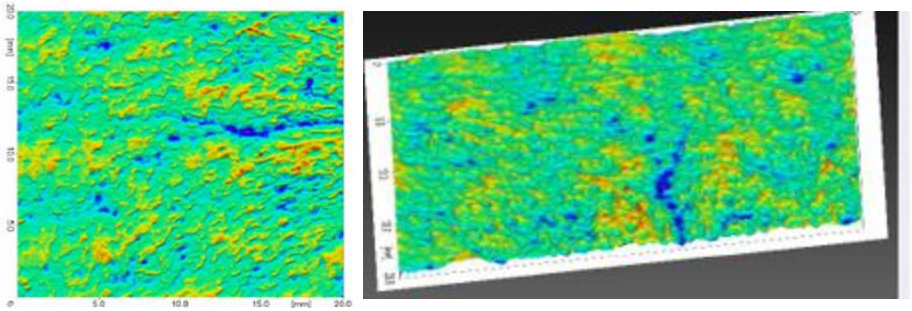
- Shows **significant smoothing & anti-wrinkle** effects by decreasing the skin relief parameters Ra and Rz by **5 %** each
- Positive effects could be measured in **66-68 %** of the volunteers
- Both the placebo and the comparator products had no significant effect

Skin refining – RonaCare® Luremin®
Visible skin smoothing effect

D0



D28



RonaCare® Luremin® visibly reduces fine lines and wrinkles

Exemplary pictures and 2D/3D-Dermascan® values

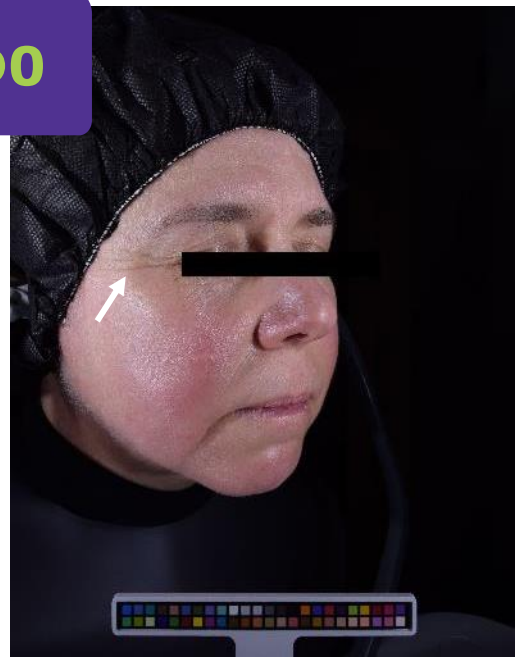
51 yr old woman, D28-D0

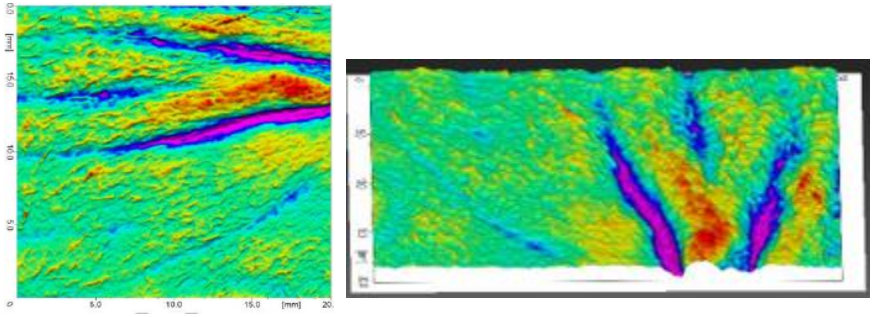
Ra: -8.4 µm (-21 %)
Rz: -20.1 µm (-14 %)
Rt: -38.0 µm (-15 %)

Skin refining – RonaCare® Luremin®
Visible skin smoothing effect

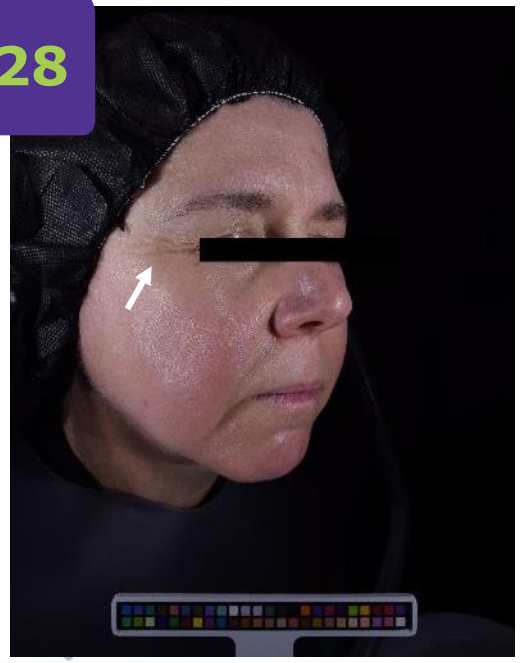
RonaCare® Luremin® visibly reduces fine lines and wrinkles

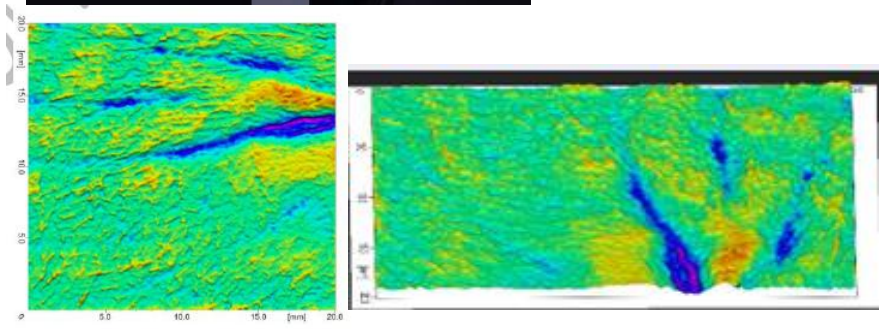
D0





D28





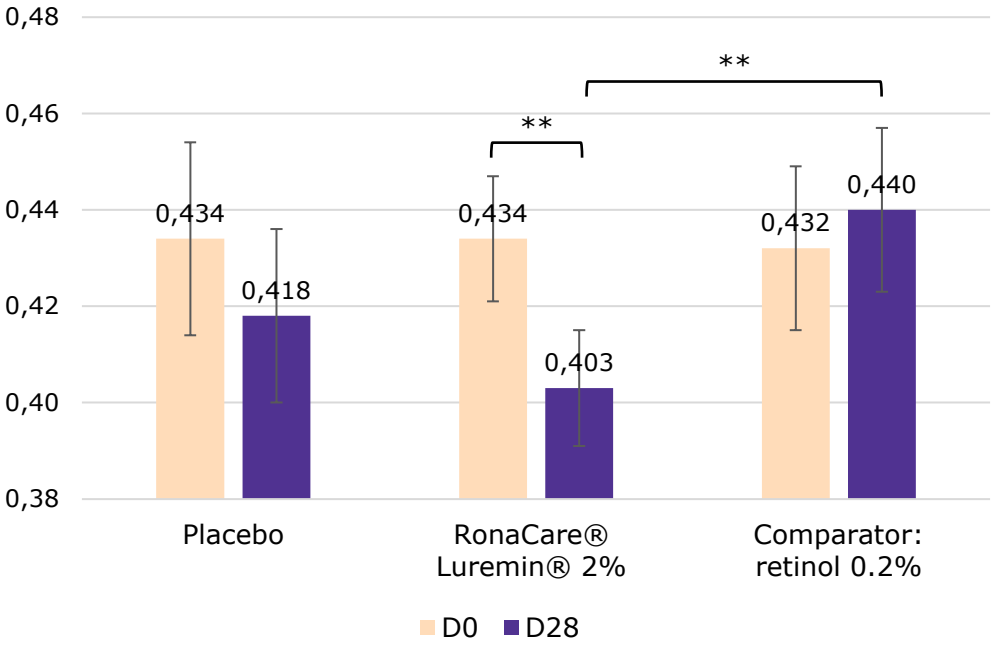
Exemplary pictures and 2D/3D-Dermascan® values

47 yr old woman, D28-D0

Ra: -18.6 µm (-30 %)
Rz: -75.4 µm (-30 %)
Rt:-166.5 µm (-33 %)

Skin refining – RonaCare® Luremin®
Skin firming

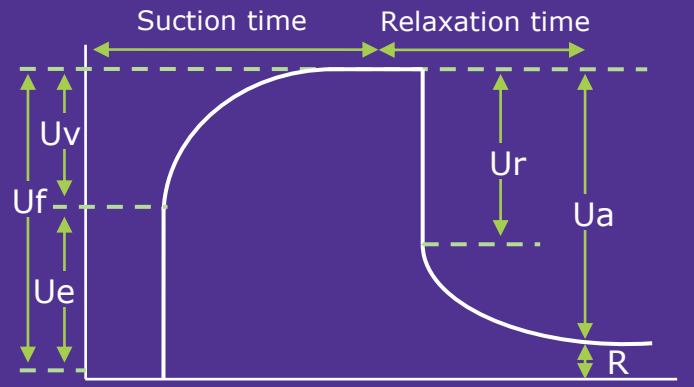
Skin firmness R0 (mm)



+7 %
skin firmness
with RonaCare® Luremin® 2%

** $p<0.01$ (ANOVA)

The skin's biomechanical properties can be evaluated using Cutometer® (Courage+Khazaka, Germany). The **cutaneous firmness** is linked to the maximal deformation amplitude. A **decrease in R0** characterizes a **skin firming** effect.



Uf: final deformation (firmness)
Ue: immediate extensibility
Uv: delayed distension (viscoelasticity)
Ur: immediate retraction (tonicity)
Ua: total recovery of initial state
R: residual deformation

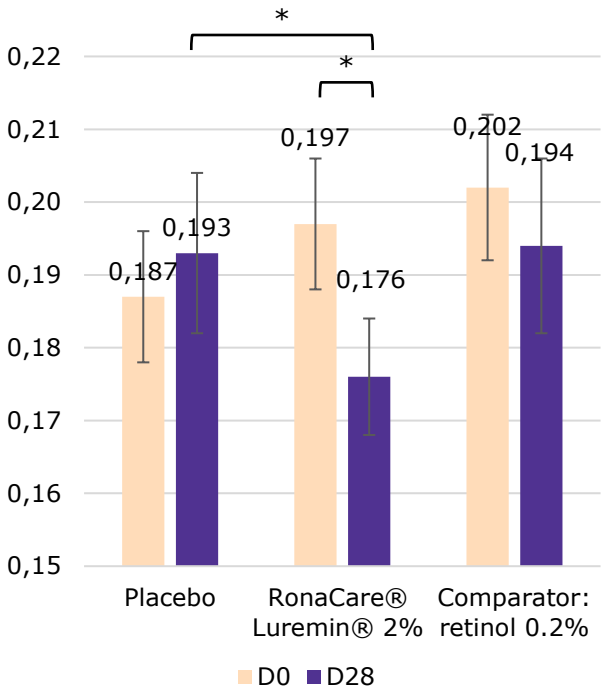
R0= Uf
R1= Uf-Ua
R5= Ur/Ue

RonaCare® Luremin®

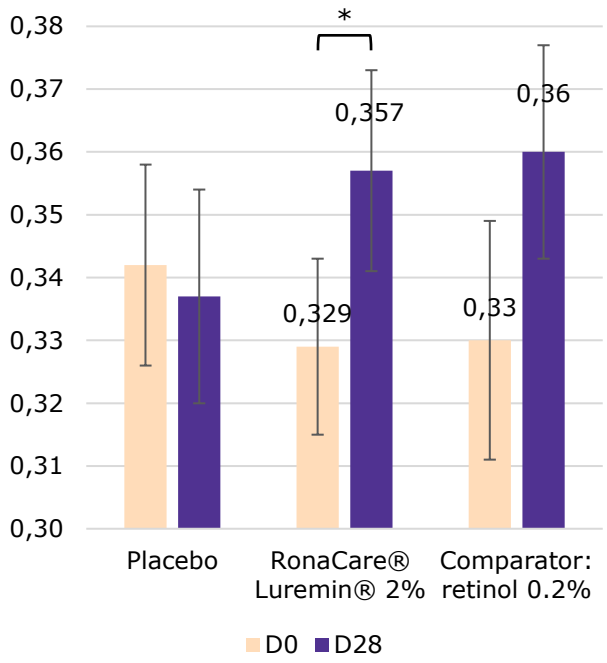
- Shows a **significant firming** effect as it decreased R0 by **7 %;** Positive effects could be measured in **70 %** of the volunteers
- In addition, it improves skin firmness **significantly better** than the retinol product
- Both the placebo and the comparator products had no significant effect

Skin refining – RonaCare® Luremin®
Skin elasticity

Skin elasticity R1 (mm)



Net elasticity R5 (mm)



+11 % skin elasticity, **+9 % net elasticity**
with RonaCare® Luremin® 2%

* $p<0.05$ (ANOVA)

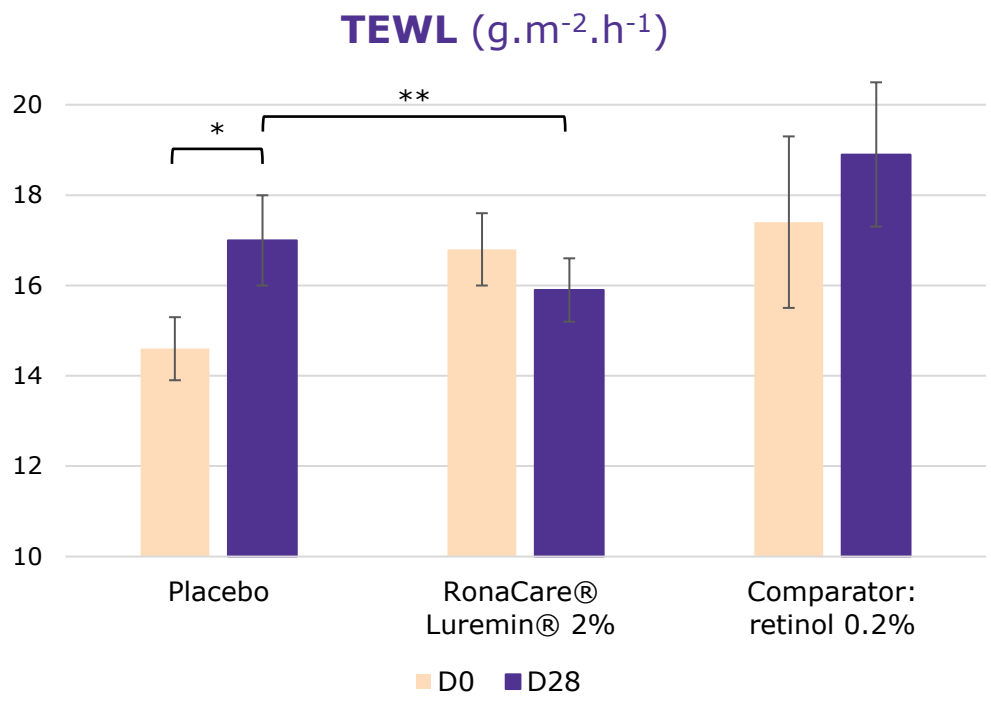
R1 (Uf-Ua): Elasticity
Skin capacity to return to its initial state. A **decrease in R1** characterizes a **more elastic, younger behaving skin.**

R5 (Ur/Ue): Net elasticity
Ratio of immediate retraction (Ur) to immediate extensibility. An **increase in R5** characterizes a **more elastic skin.**

RonaCare® Luremin®

- Significantly **increases skin elasticity** as shown by the decrease in R1 by **11 %** and the increase in R5 by **9 %**
- In addition, it improves skin elasticity **significantly better** than the placebo
- Both the placebo and the comparator products had no significant effect

Skin refining – RonaCare® Luremin®
Skin barrier integrity



Transepidermal water loss (TEWL) is related to the state of the skin barrier. A low TEWL is associated with good skin barrier integrity, which allows a balanced mechanism for regulating skin water exchange.

RonaCare® Luremin®

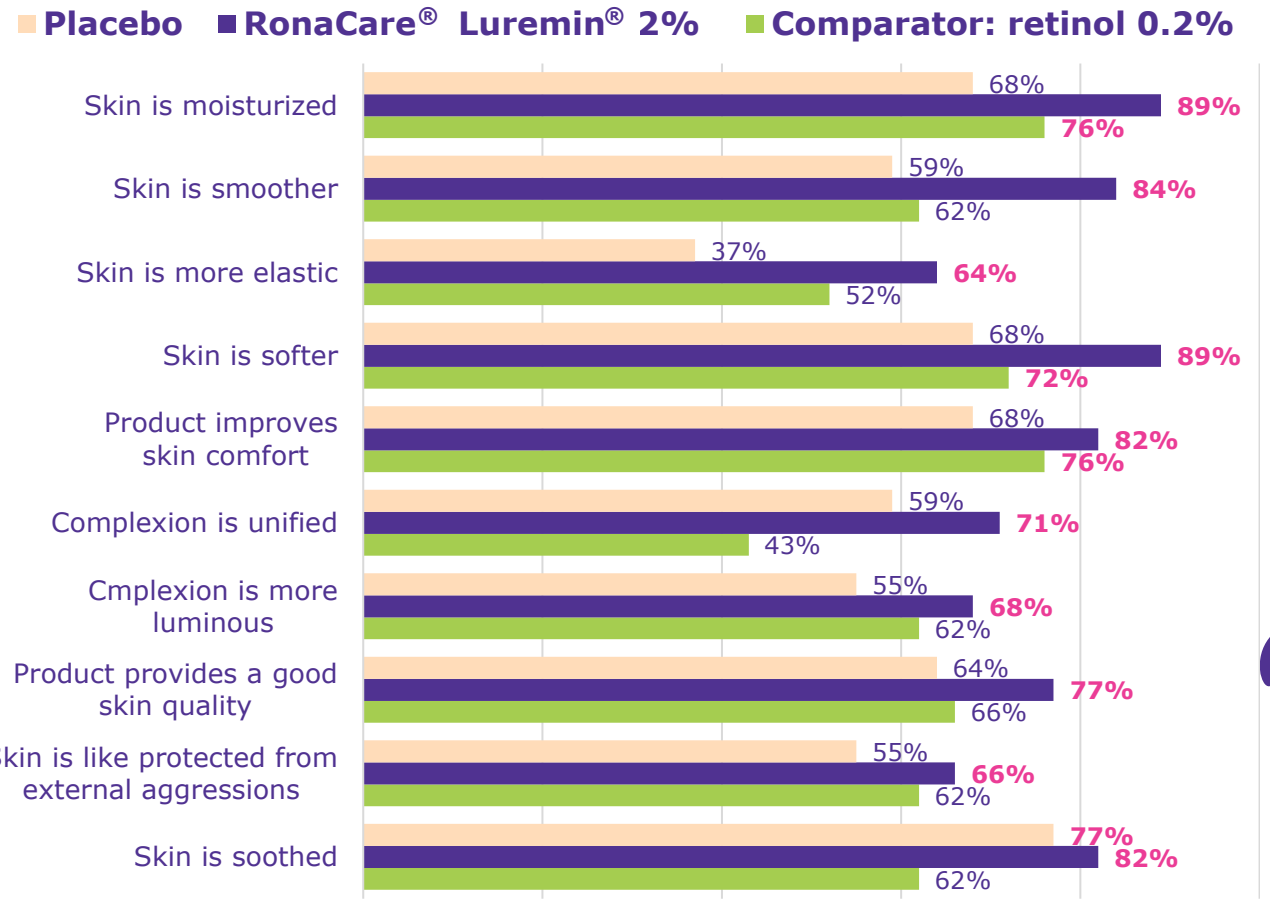
- Fully **compensates** the significant increase in TEWL induced by the placebo product (+16 %)
- This correlates with the built up of **involucrin** and **transglutaminase 1** induced by DHMC
- Both the test product with **RonaCare® Luremin®** and the comparator are able to **maintain the integrity** of the cutaneous barrier (no significant variation after 28 days of use)

SKIN BARRIER
maintenance
with RonaCare® Luremin® 2%

* $p<0.05$ (ANOVA)

Skin refining – RonaCare® Luremin®
Subjective evaluation survey

Product efficacy after 28 days of use (% of satisfied subjects)



The test product containing RonaCare® Luremin® was found to be more effective in improving various skin parameters compared to the placebo and the retinol-containing product

In pink: Significant proportion of positive answers

Back to efficacy overview

SKIN REFINING

RONACARE® LUREMIN®
Dihydroxymethylchromone

SKIN STRUCTURE

Increased gene expression of several matrix proteins (collagens, elastin, laminin) + proven **boost of collagen synthesis** support a dense protein network in the extracellular matrix

SKIN SMOOTHING

Significant **smoothing and wrinkle-reducing** effects demonstrated in two placebo-controlled clinical studies

Skin's state improvements confirmed by the volunteers in subjective evaluations

Better performance than retinol in a comparative efficacy test

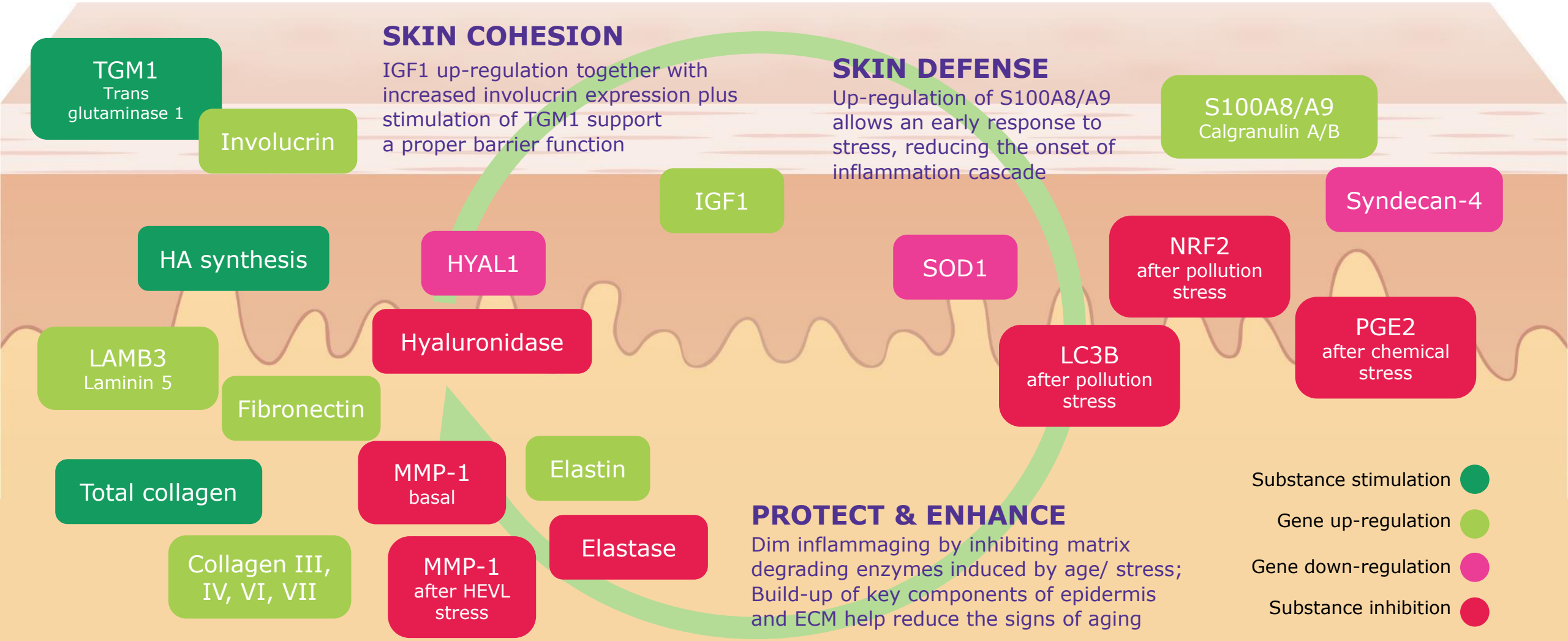
MERCK

04

RonaCare® Luremin®

MODE OF ACTION

Support – Reduce – Protect & Enhance
The 3-level working power of RonaCare® Luremin®



RonaCare® Luremin® / DHMC
Support – Reduce – Protect & Enhance

SUPPORT the barrier function

- Up-regulation of involucrin and IGF1
- Increased TGM1 activity

PROTECT & ENHANCE the extracellular matrix

- Decreased MMP-1 release, also under Blue light stress
- Inhibition of elastase
- Down-regulation of HYAL1 & inhibition of hyaluronidase
- Stimulation of HA synthesis
- Up-regulation of collagen III,IV, VI,VII
- Stimulation of collagen synthesis
- Up-regulation of laminin
- Up-regulation of fibronectin

REDUCE stress-induced signs of inflammation

- Decreased PGE2 release (chemical stress)
- Decreased activation of biomarkers of oxidative stress Nrf2 and LC3B (pollution stress)
- Up-regulation of calgranulin A & B; Down-regulation of syndecan-4, SOD1

3-level working power

Support – Reduce – Protect & Enhance
The 3-level working power of RonaCare® Luremin®

SUPPORT

The **skin barrier** is improved by the increased built up of involucrin and transglutaminase 1

REDUCE

DHMC reduces **signs of inflammation** by reducing irritant and ROS induced stress signals (e.g., pollution) – mechanisms typically increased upon stimulation like autophagy (LC3B) or the transcription factor NRF2 are not activated

PROTECT & ENHANCE

Degrading enzymes like hyaluronidase, MMP-1, elastase are reduced or not upregulated after blue light stress (MMP-1) and thus do not contribute to chronical and stressed-induced aging **"inflammaging"**

Upregulation of important proteins of the extracellular matrix like collagens III, IV, VI, VII, laminin, elastin, and increase of **collagen production** help densify the skin and reduce the signs of aging

RonaCare® Luremin®

Visibly **smoother, refined** skin appearance & **more supple** skin.

CONTACT DATA

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