20th Congress of the European Society for Photobiology

Lyon 2023

In vivo biomarkers study to evaluate photoprotection products

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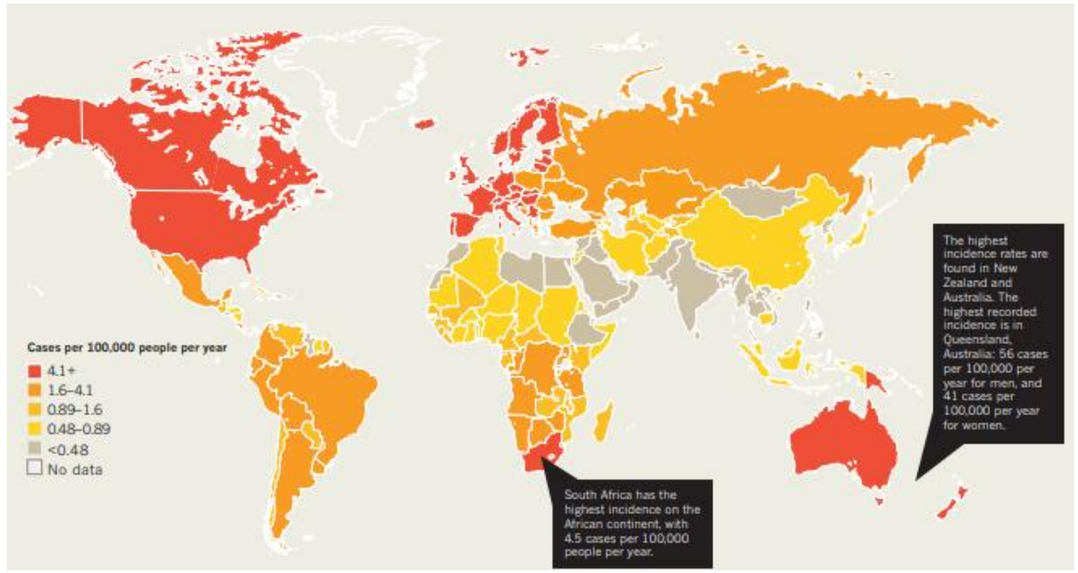
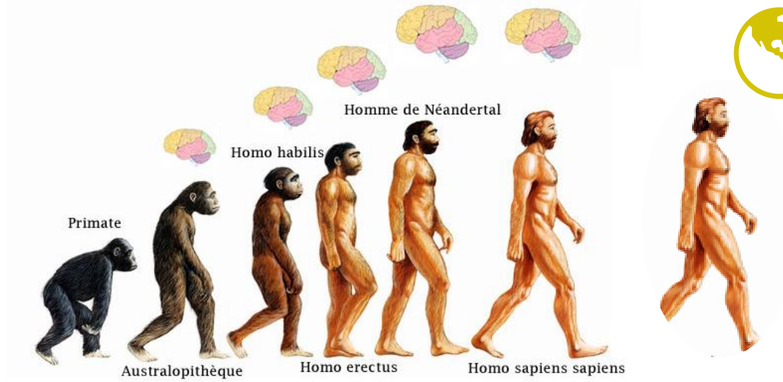
Sun and human skin...

- Most frequent cancers in white population concern the skin
- Their incidence increase every year for 20 years
- The highest rate of melanoma occurs where people are predominantly light skin
- More than 80% of skin cancers are linked to excessive exposure to sun UV (IARC, 2018)

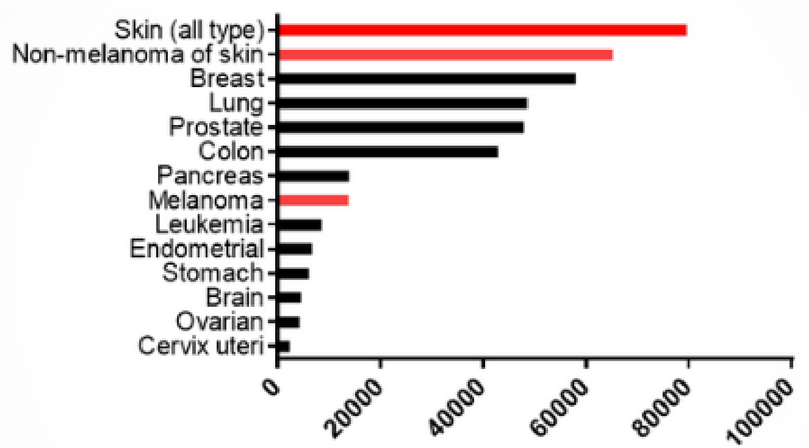


Holmes, D. The cancer that rises with the Sun. *Nature* **515**, S110–S111 (2014)



Source : www.histoire-du-monde.fr

Number of new cancers cases per year in France



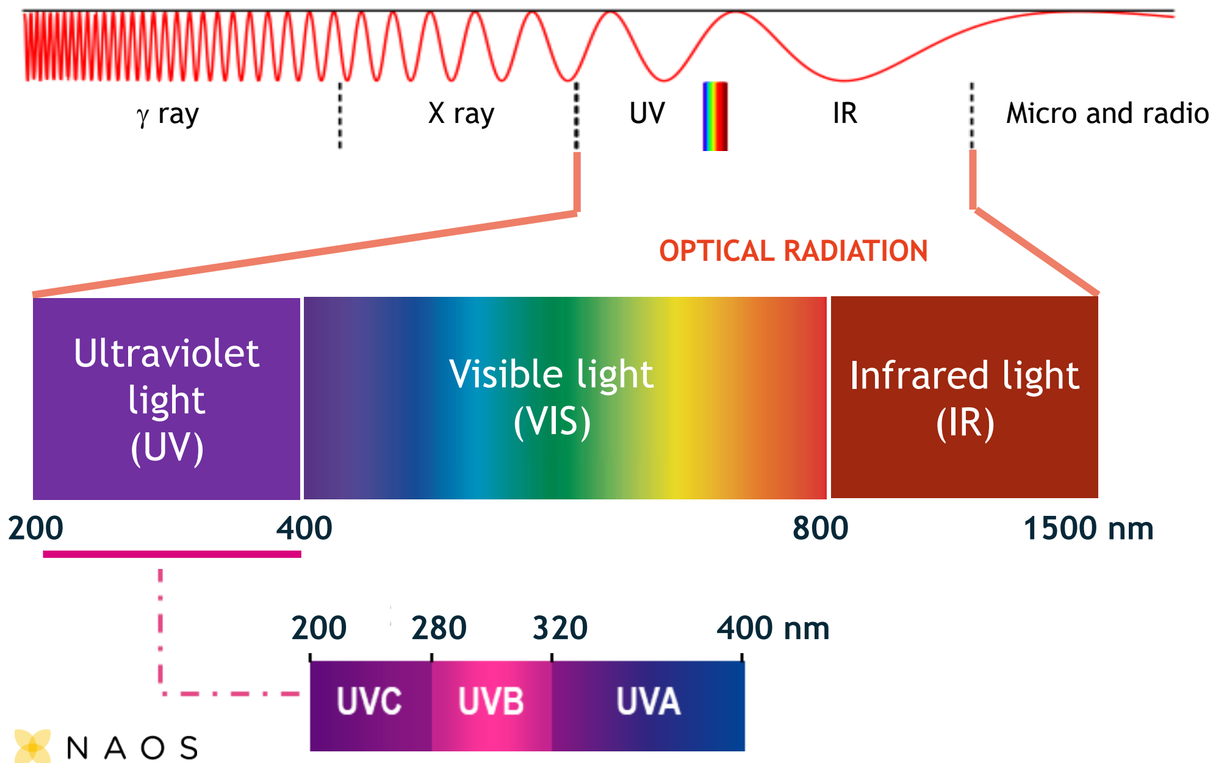
Source : Fondation pour la Recherche Médicale (www.frm.org)

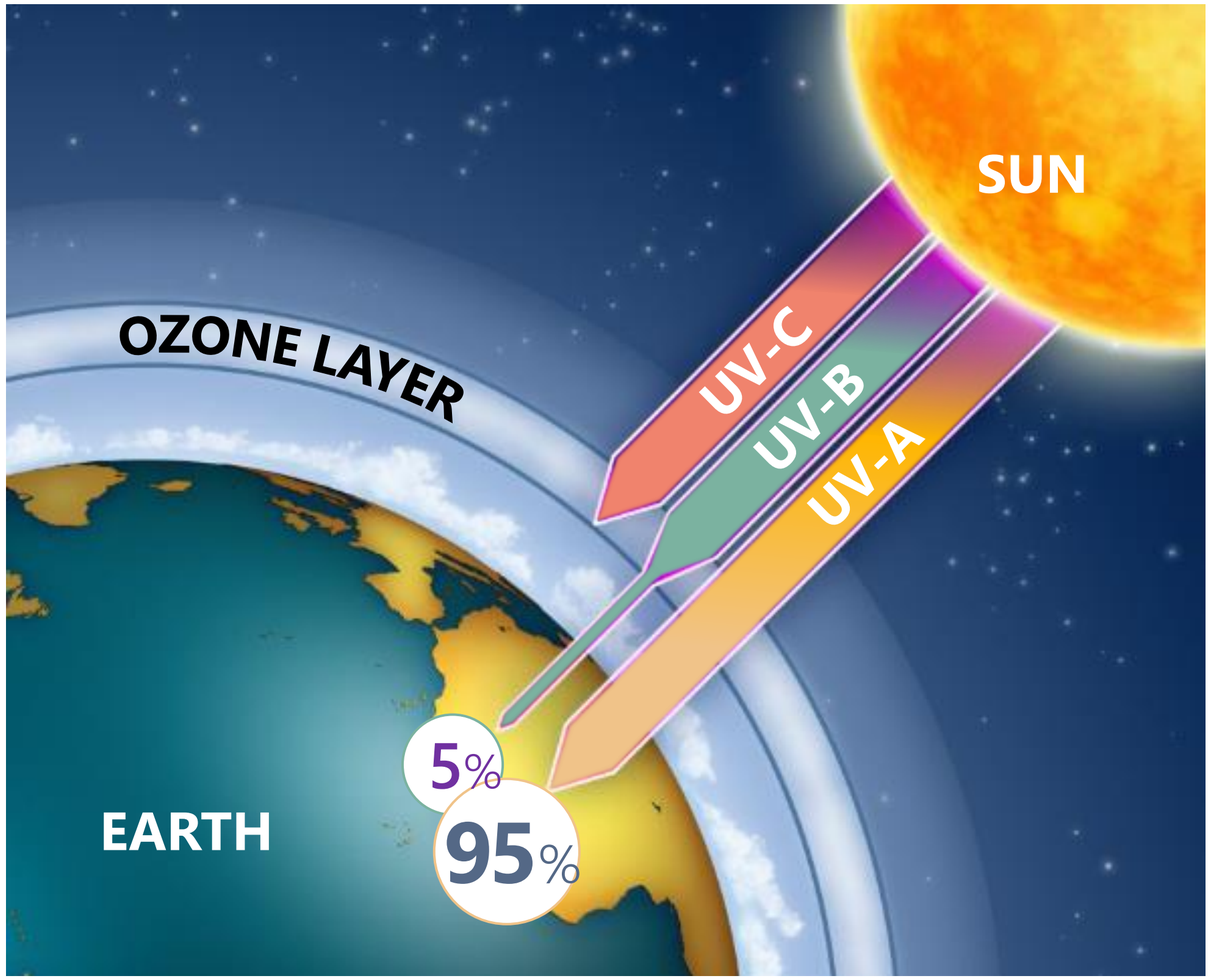
- Holmes et al., 2024
- Leiter et al., 2015
- IARC (International Agency for Research on Cancer, 2018)

Solar spectrum on earth

The solar spectrum is composed of different electromagnetic waves.

The UV spectrum is composed of **UVB and mainly UVA** (95% of UV).





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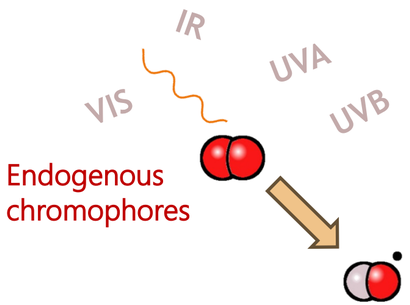
Optical radiations penetrate differently in the skin

Penetration of optical radiation in the skin is wavelength dependent

UVB are stopped within the epidermis reach the superficial dermis

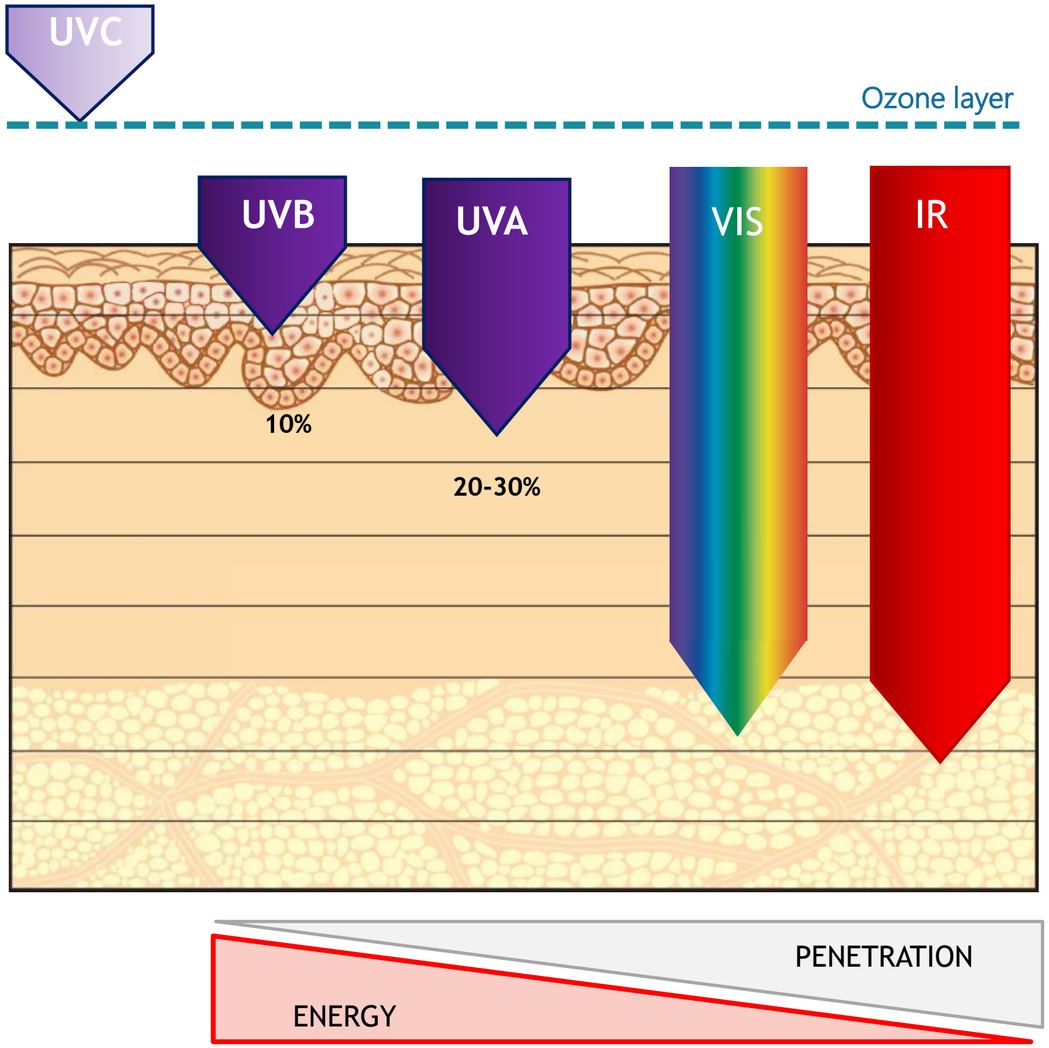
UVA, visible and IR reach the deep dermis



Radiation interacts with specific endogenous chromophores

--> activation of specific signaling pathways activation
--> ROS

--> cutaneous biological effects



- Schroeder *et al.*, 2024
- Piel *et al.*, 2011
- Svobodova *et al.*, 2016

Biological effects of UV on skin

POSITIVE EFFECTS

NEGATIVE EFFECTS

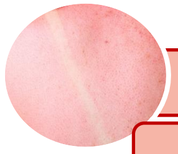
MELANOGENESIS

VITAMIN D SYNTHESIS

WELL-BEING

KILLING PATHOGENS

BARRIER FUNCTION



INFLAMMATION

ACTINIC ERYTHEMA

PROTEINS ALTERATION

ECM DEGRADATION

LIPIDS OXIDATION

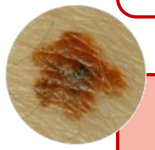
ROS

PHOTO-AGING

DNA DAMAGE

PHOTO-IMMUNOSUPPRESSION

ABNORMAL CELL PROLIFERATION



SKIN CANCERS*

INFLAMMATORY PATHOLOGIES RESOLUTION

* IARC 2009

• Holick *et al.*, 2016

UVA and UVB have different biological effects

280nm 320nm 400nm 800nm

UVB | UVA | VISIBLE

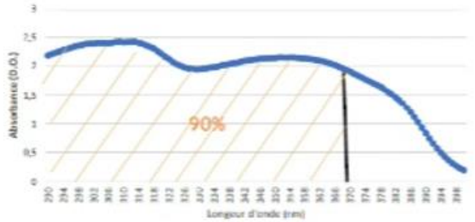
MOLECULAR CELLULAR EFFECTS

- DNA damage
- Oxidative DNA damage
- Sun burn
- Free radical generation
- Vitamine D
- Microbiome modulation ?

SKIN EFFECTS

- Actinic erythema
- Pigmentation
- Immediate pigmentation
- Immunosuppression
- Photoaging
- Cancers

Suncare products and reglementory evaluation



Sun Protection Factor (SPF)
<u>UVB</u> + UVA

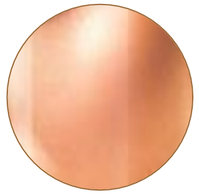
SPF *in vivo*
- ISO 24444 : 2019 / Amdt 2022
- AS / NZS : 2021
- FDA 2021 (USA/CA)

ERYTHEMA

UVA Protection Factor (UVA-PF)
UVA

UVA *in vivo* : PPD (Persistant Pigment Darkening)
- ISO 24442 : 2022

UVA *in vitro*
- ISO 244432021 : *in vitro* UVA + Critical wavelength (CW)
- FDA 2021 (USA/CA) : Critical wavelength (CW)



IMMEDIATE PIGMENTATION

Water resistance *in vivo* (Optionnal)

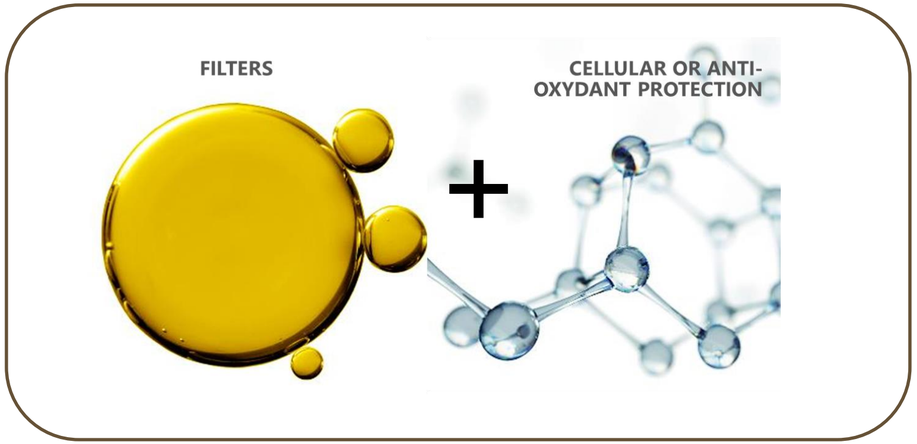
- ISO 16217 : 2020
- ISO 18861 : 2020
- FDA 2021 (USA/CA)
- AS/NZS 2604 : 2021

Other non-standard methods for specific claims

- Photo-resistance
- Water resistance
- Sand resistance
- Humidity resistance

At the reglementory level, evaluation of suncare products concern effects of UVB and UVA based on measure of erythema and immediate pigmentation

Photo-protection : suncare products



Organic or mineral filters

+

Anti-oxidant
cellular protection

280nm 320nm 400nm 800nm

UVB | UVA | VISIBLE

MOLECULAR CELLULAR EFFECTS

- DNA damage
- Oxidative DNA damage
- Sun burn
- Free radical generation
- Vitamine D
- Microbiome modulation ?

SKIN EFFECTS

- Actinic erythema
- Pigmentation
- Immediate pigmentation
- Immunosuppression
- Photoaging
- Cancers

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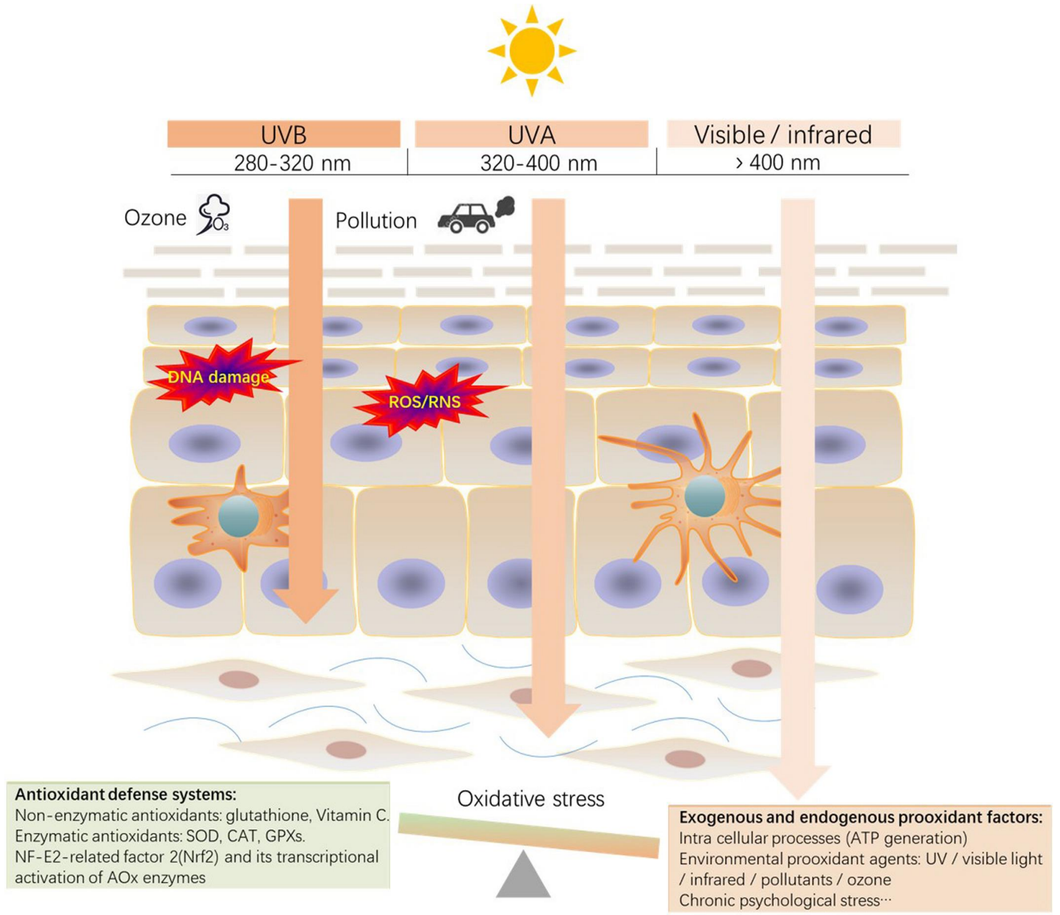
UV rays induce ROS and antioxidant defense systems



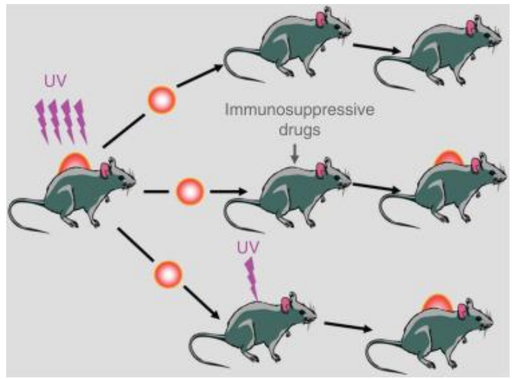
Anti-oxidant defense systems		
Non enzymatic	Enzymatic	Transcriptional factor
Vitamins C, E Glutathion Squalene	SOD Catalase Glutathion peroxydase	NRF-2

- Revue Chen *et al*. 2021

UV rays induce immunosuppression

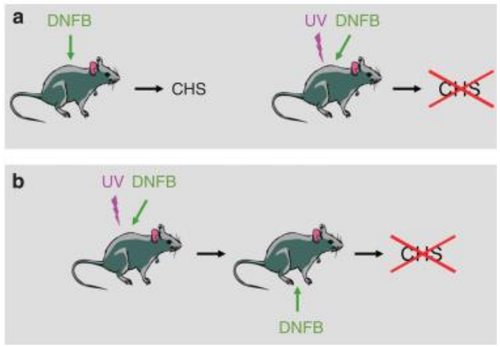
- UV radiation inhibits rejection of transplanted skin tumor

Kripke et al., 1977

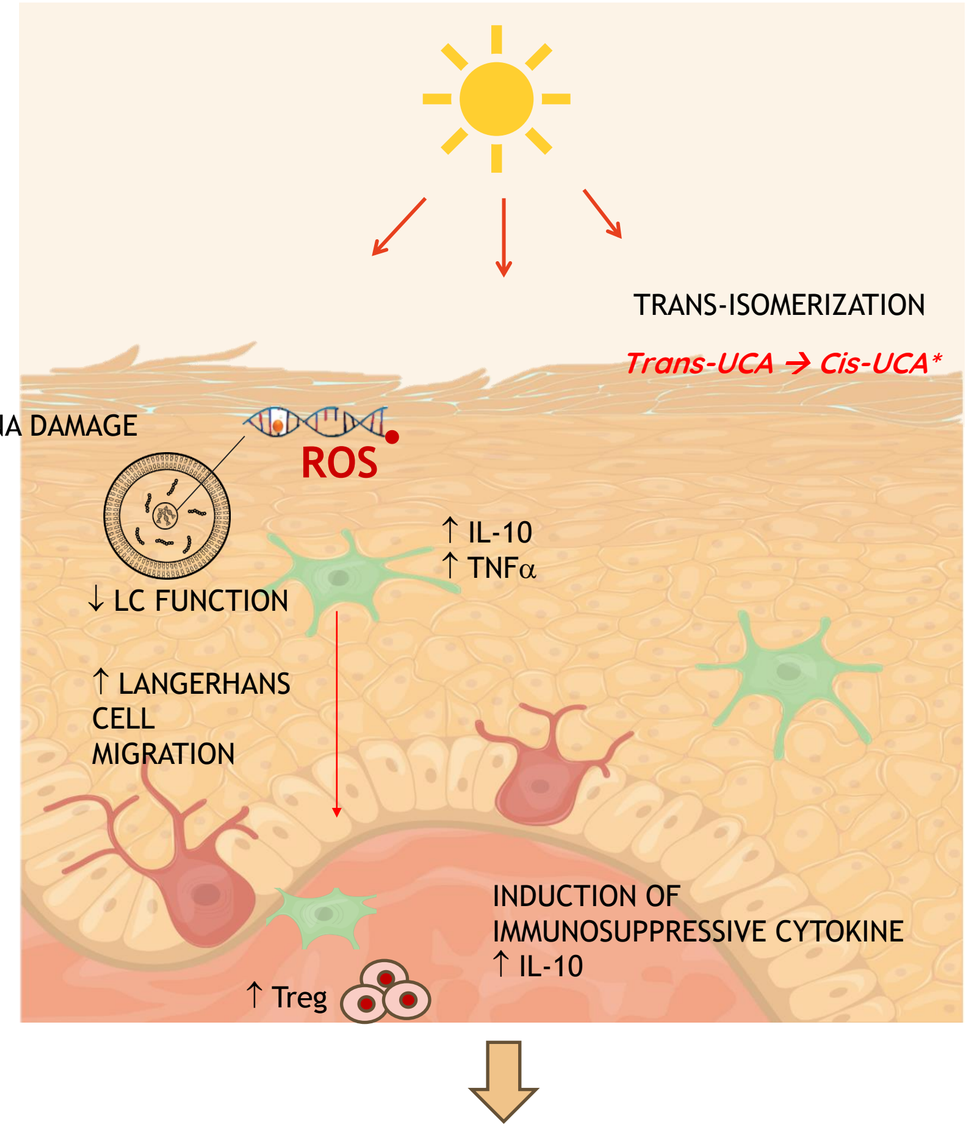


- UV radiation inhibits sensitization and contact hypersensitivity induced by DNFB

Toews et al., 1980



Figures from Schwarz et al.,2010



- Schwarz *et al.*, 2010
- Noonan *et al.*, 1992
- Reeve *et al.*, 1989

UV rays impact function barrier in human skin

- *Stratum corneum* and epidermis are a major protective barrier for solar radiation (keratin, lipids, amino-acids, UCA)

- **Suberythemal doses of UV appear to have positive effects** on epidermal barrier function
  --> thickness increase of *stratum corneum*

- **Erythemal doses and chronic exposition have negative impact :**
  - ↑ TEWL
  - ↓ *stratum corneum* hydration
  - Modification of lipids organization
  - Alteration of tight junction
  - Modification of amino acids

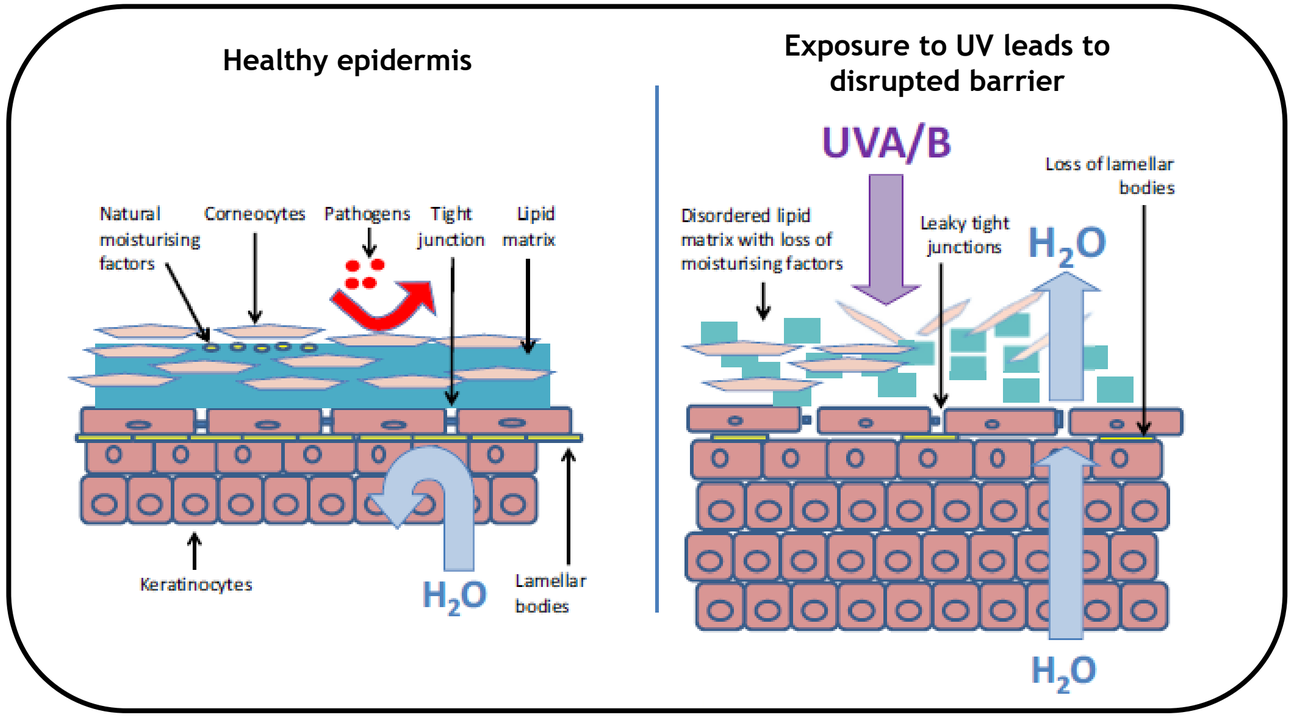


Figure from Alhasaniah *et al.*, 2018

- Kim et al., 2015
- Lim et al., 2018
- Jin et al., 2016
- Wefers *et al.*, 1991
- Alhasaniah *et al.*, 2018
- Yoon et al., 2019

UV rays alter skin microbiome

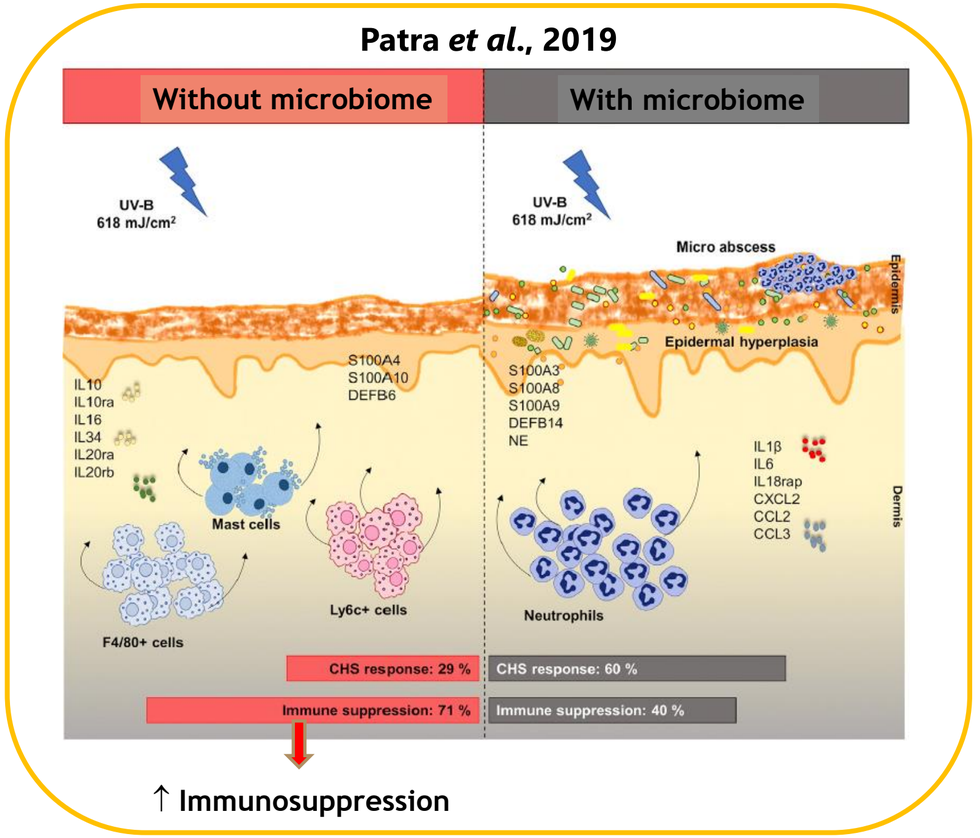
Research is ongoing….

Effect of UV on skin microbiome

- UV alters skin microbiome
- UV has a cytotoxic effect on skin microbiome
- UV modifies microbiome composition

Roles of microbiome

- Protects against immunosuppression UV-induced
- Regulates immunity
- Modulates skin metabolomic and lipidomic profiles of skin



- Burns *et al.*, 2018
- Patra *et al.*, 2019
- Rai *et al.*, 2022
- Dotterud *et al.*, 2008
- Patra *et al.*, 2023

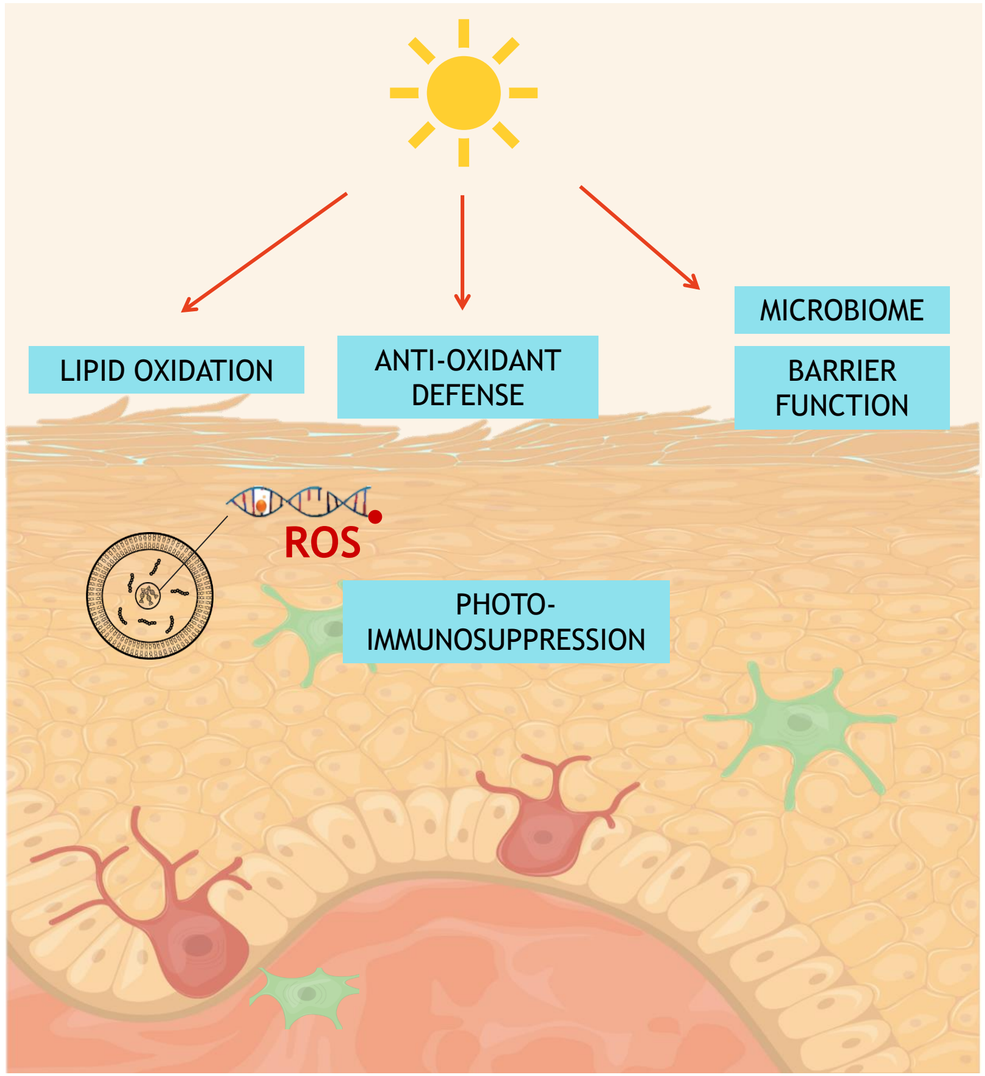
Non-invasive skin biomarkers modulated by UV

Categories	Biomarkers	Irradiation	Results	References
Barrier function	**Desmogleine-1**	Sampling then UVB	dispersion of desmogleine-1	Lipsky *et al.*, 2019
Hydration	**MNF**	UV (290 à 400nm) 0.5, 1, ou 1.5 MED	↑ alanine, glycine, histidine, ornithine, serine à 0h ↑ Ornithine à 24h ↑ Alanine, histidine, ornithine, serine 72h ↓ Urea 0, 24 and 72h ↓ Lactate à 0 et 24h	Yoon *et al.*, 2019
Oxidative damage	**Keratin oxidation**	UVA + UVB	↑ Keratin oxidation (methionine)	Lee *et al.*, 2016
Oxidative damage	**Protein carbonylation**	UVA	↑ Carbonylated proteins	Cho *et al.*, 2021
Oxidative damage	**squalene**	UVA	↑ Squalene oxidation	Ekanayake-Mudiyanselage *et al.*, 2004
Barrier function	**Lipids**	UVA or UVB reapeted irradiation	↑ Total lipids (triG, acides gras, squalene, ceramides)	Wefers *et al.*, 1991
Barrier function	**Lipids**	UVA + UVB	↓ Ceramide and cholesterol	Yoon *et al.*, 2019
Inflammation	**IL-1RA**	UVB	↑ IL-1RA → IL-1α	Hirao *et al.*, 1996
Immuno-suppression	**UCA**	UVB + UVA	↓ trans-UCA	Yoon *et al.*, 2019
Immuno-suppression	**UCA**	UVB or UVA	↑ cis-UCA	Krien *et al.*, 1994
Oxidative response	**DJ-1**	UVB	↑ DJ-1	Ischiwatari *et al.*, 2015
Extra-cellular Matrix (MEC) degradation	**MMPs**	UVB	↑ MMP-2 ↑ MMP-9	Takada *et al.*, 2006

- Only few non-invasive biomarkers modulated by UV are described
- Biomarkers associated to oxidation, barrier function / hydration, immunosuppression and MEC degradation

Aim of our study

- Better understand effects of UV on human skin
- Detect pertinent non invasive biomarkers
- Improve and analyse other photo-protection benefits
- Analyse the effects of sun care products : complementary efficacy of sun filters with active ingredients



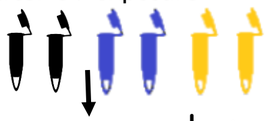
In vivo experimental design

Summary

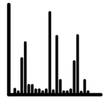
UV exposure - + +

Ectoine 0.1%
Mannitol 0.1%
SPF30 + - - +

Sampling was done 24h after UV exposure



Analysis



Stratum corneum :
- Catalase activity (Resorufin fluorescence)
- Squalene (LC/MS)
- Trans-UCA (LC/MS)
- Aminogramm (LC/MS)

Microbiome
- Metaproteomic (LC-MS/MS)

Statistical analysis

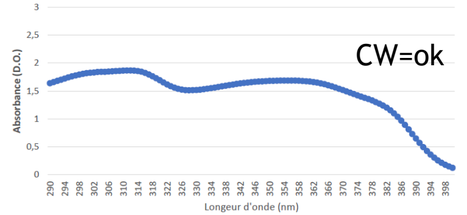
- Student's test : catalase
- Wilcoxon's test : squalene, trans-UCA and metaproteome

Inclusion criteria

- **Sex** : men (n=10)
- **Phototype** : II to III
- **Age** : 20 to 44 years (mean 27,6 years)
- **Oily skin** : sebum rate > **27 µg/cm²**
- **Exclusion** : sun exposure (3 months), tatoo, piercing, hairy back
- **During the study** : no back washing / no contact with shampoo

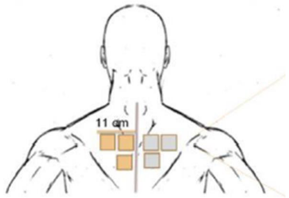
Tested products

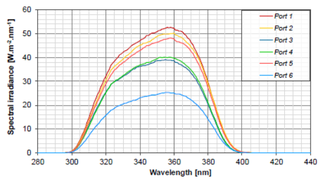
- Placebo
- Sunscreen SPF 30
- Ectoine (E) 0.1% + mannitol (M) 0.1%
- SPF 30 + E + M

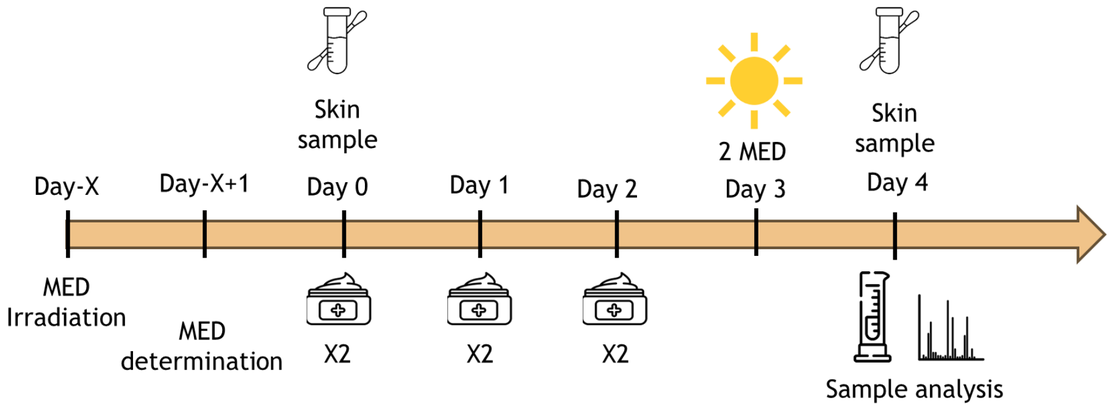


- Dose : 2 mg/cm² twice a day

Design of the study

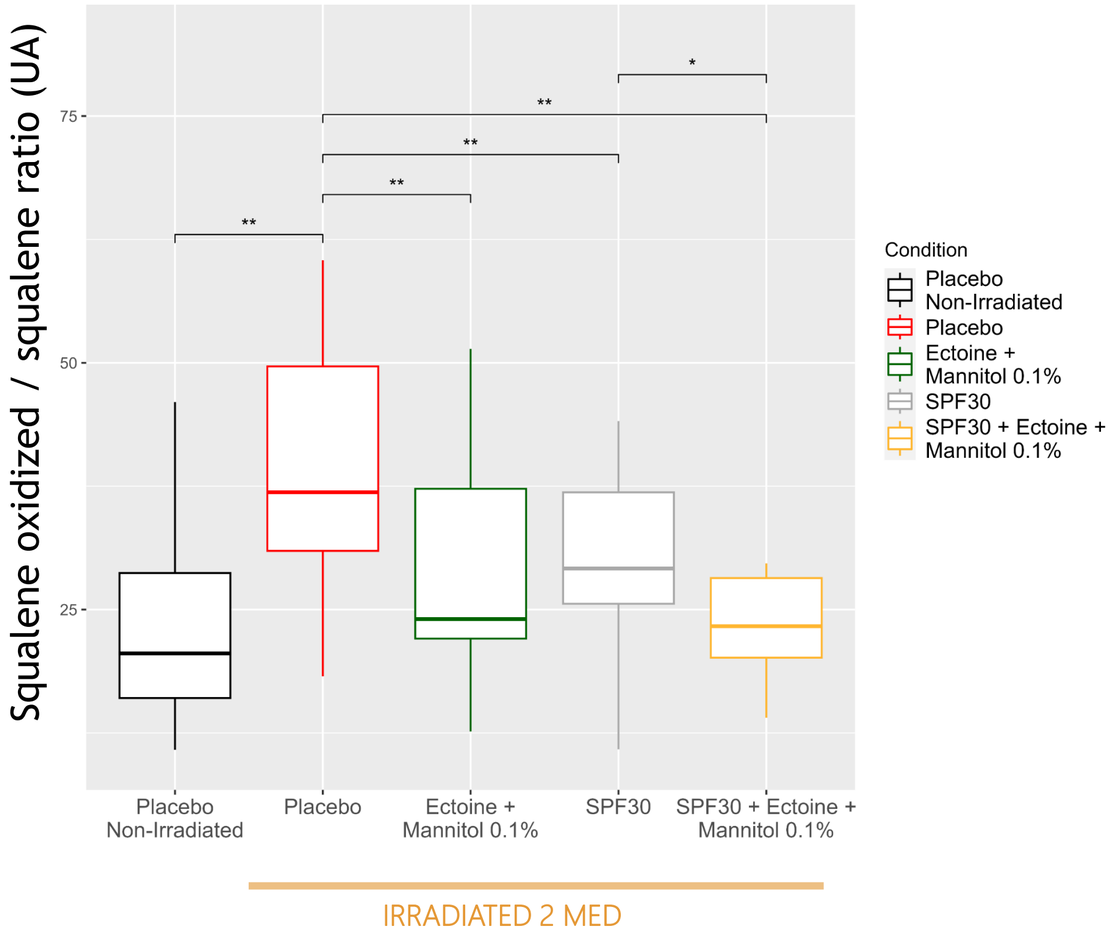




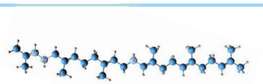


UV irradiation induces squalene oxidation *in vivo*

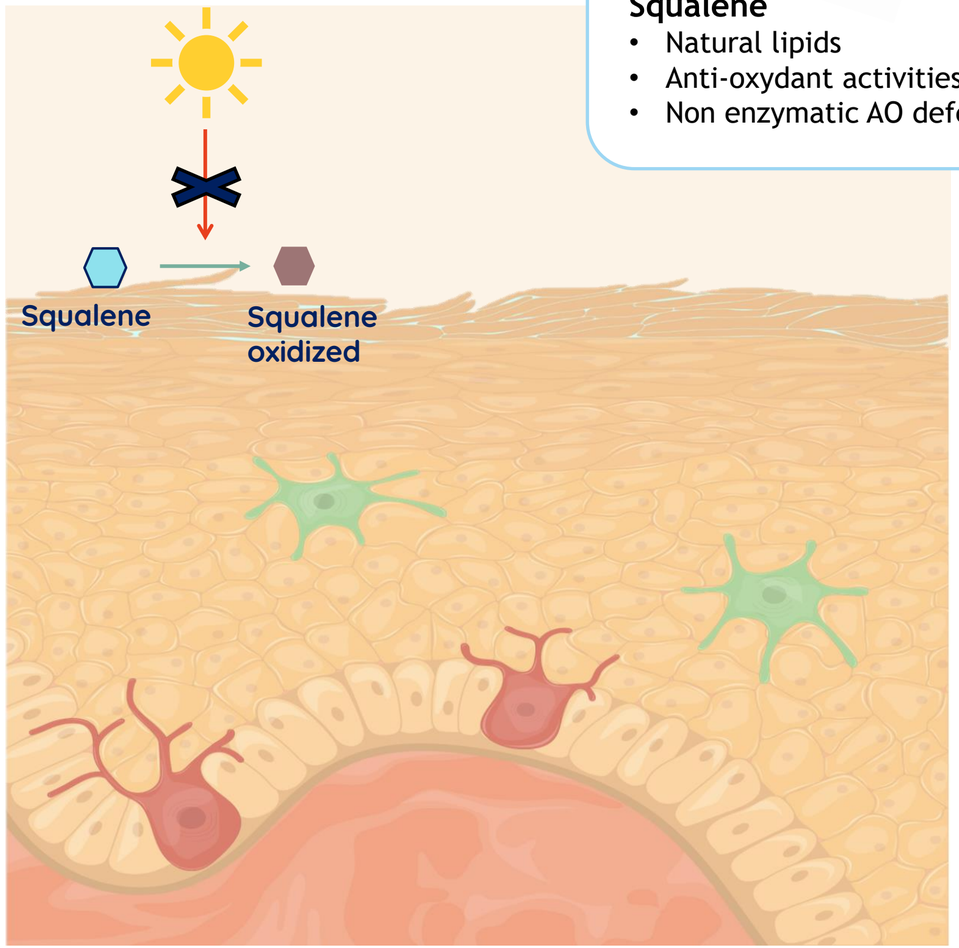
Anti-oxidant defense systems



Wilcoxon test
*p< 0.05, **p< 0.01, ***p< 0.001; ns, not significant



Squalene
- Natural lipids
- Anti-oxydant activities
- Non enzymatic AO defense

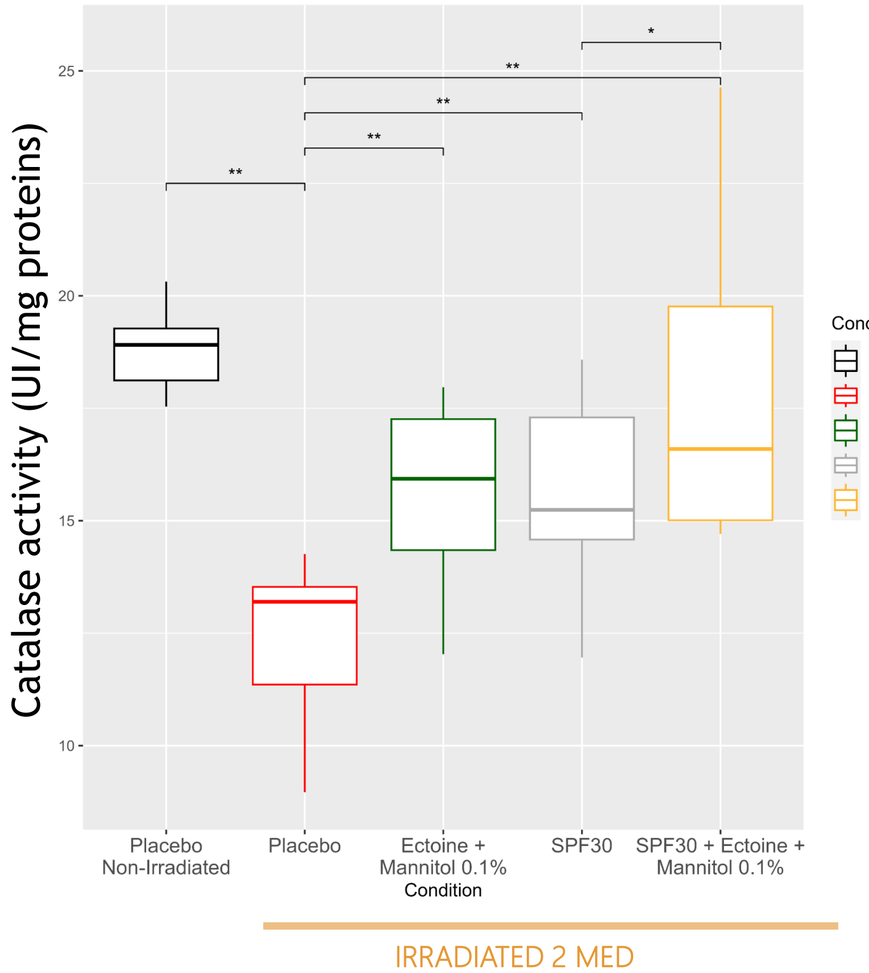


Inhibition by the products

- This sunscreen product prevents squalene oxidation with complementary effect of filters and active ingredients

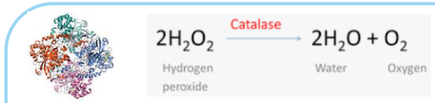
UV irradiation reduces catalase activity *in vivo*

Anti-oxydant defense systems

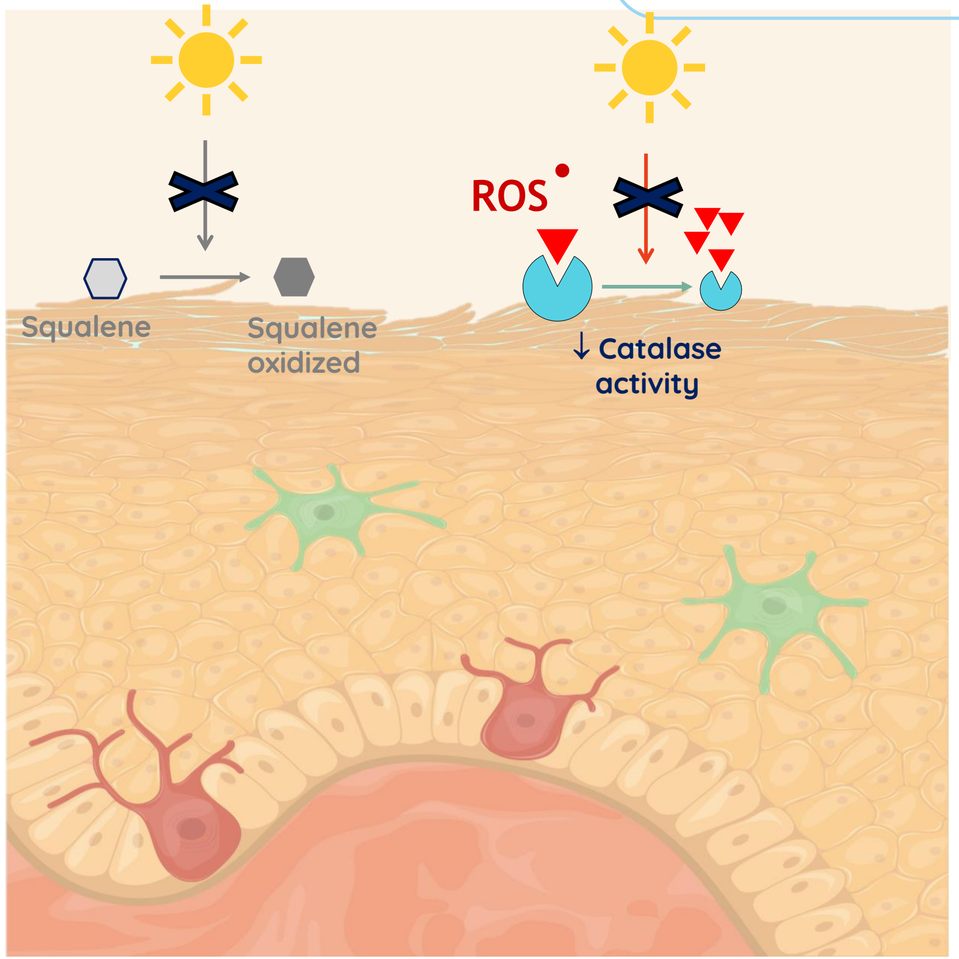


IRRADIATED 2 MED

Wilcoxon test
*p< 0.05, **p< 0.01, ***p< 0.001; ns, not significant



Catalase
- Anti-oxydant enzyme
- Heme oxidoreductase
- Dismutation of H_2O_2

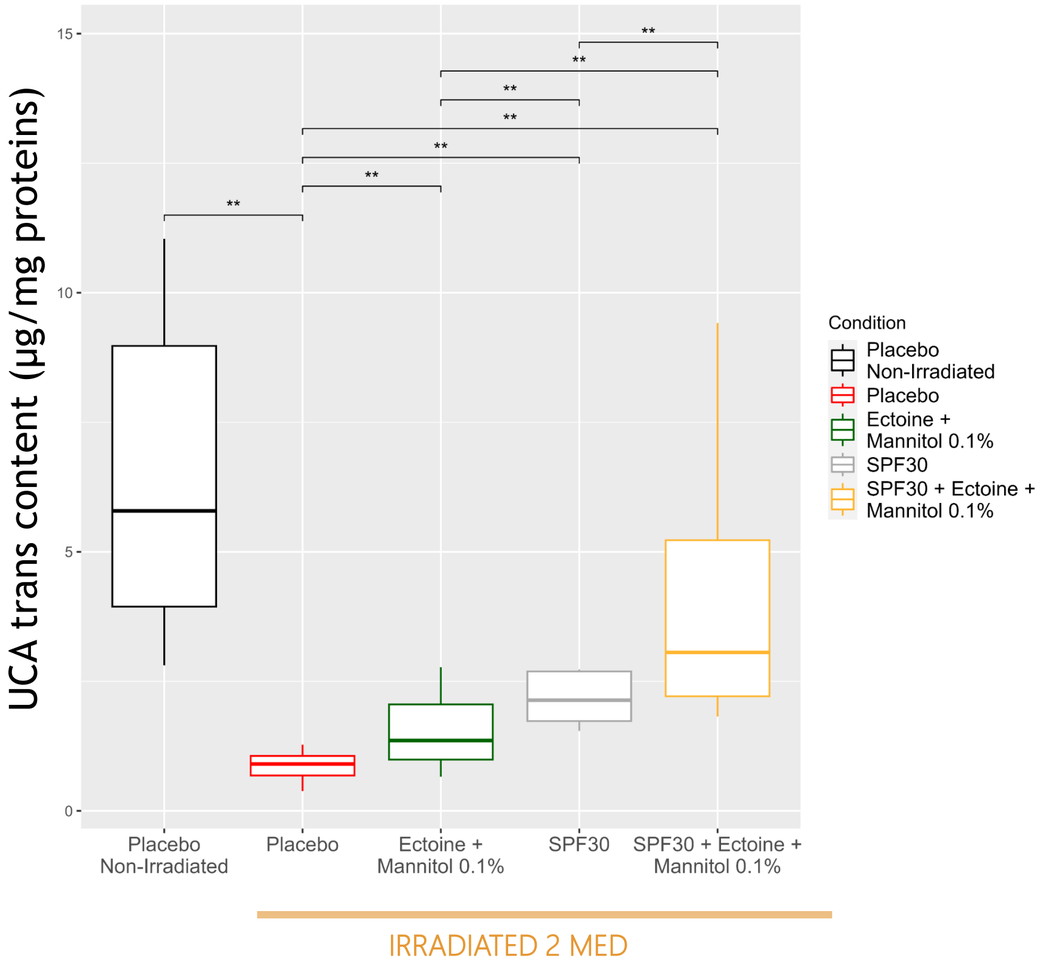


Inhibition by the products

- This sunscreen product prevents catalase inhibition activity with complementary effect of filters and active ingredients

UV irradiation reduces *trans*-UCA *in vivo* --> *cis*-UCA

Photo-immunosuppression



Wilcoxon test
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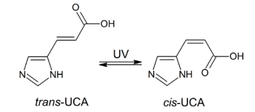
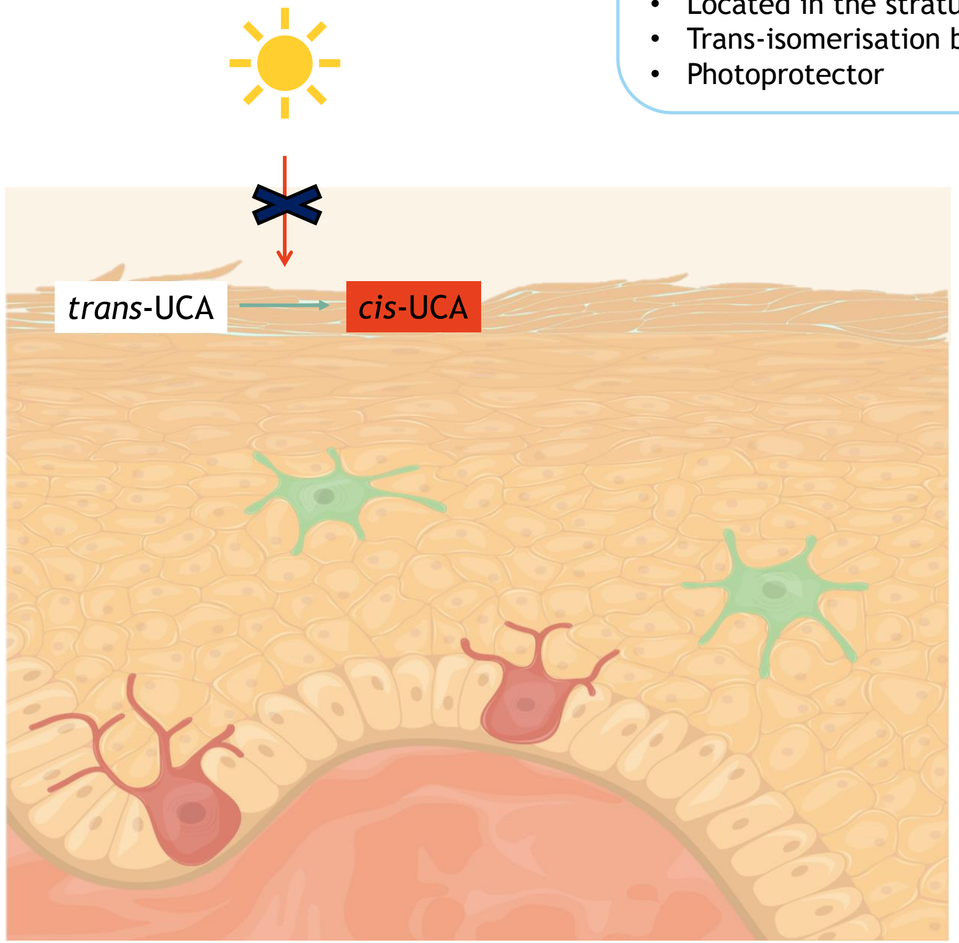


Urocanic acid (UCA)
- Metabolite of histidine
- Located in the stratum corneum
- Trans-isomerisation by UVB
- Photoprotector



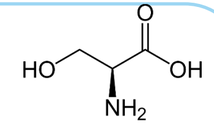
Inhibition by the products

- This sunscreen product prevents UCA trans-isomerisation with complementary effect of filters and active ingredients

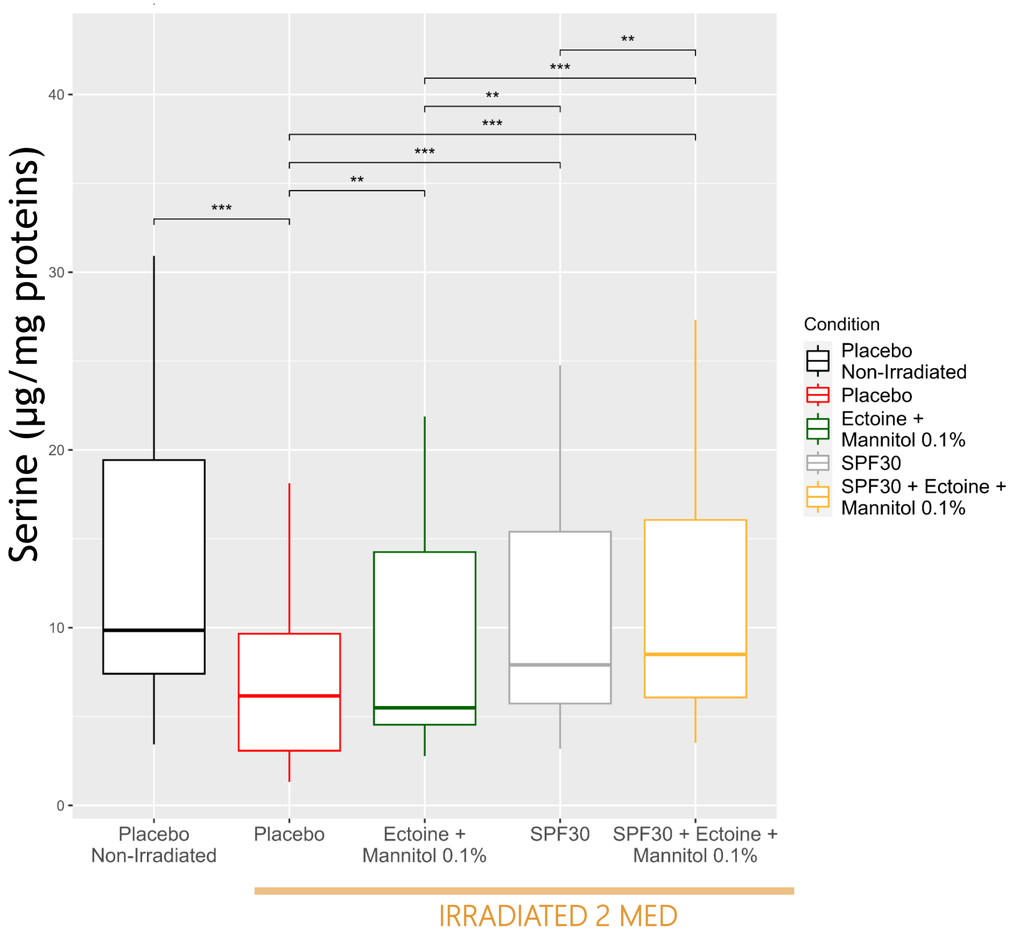
UV irradiation reduces serine content *in vivo*

Barrier function

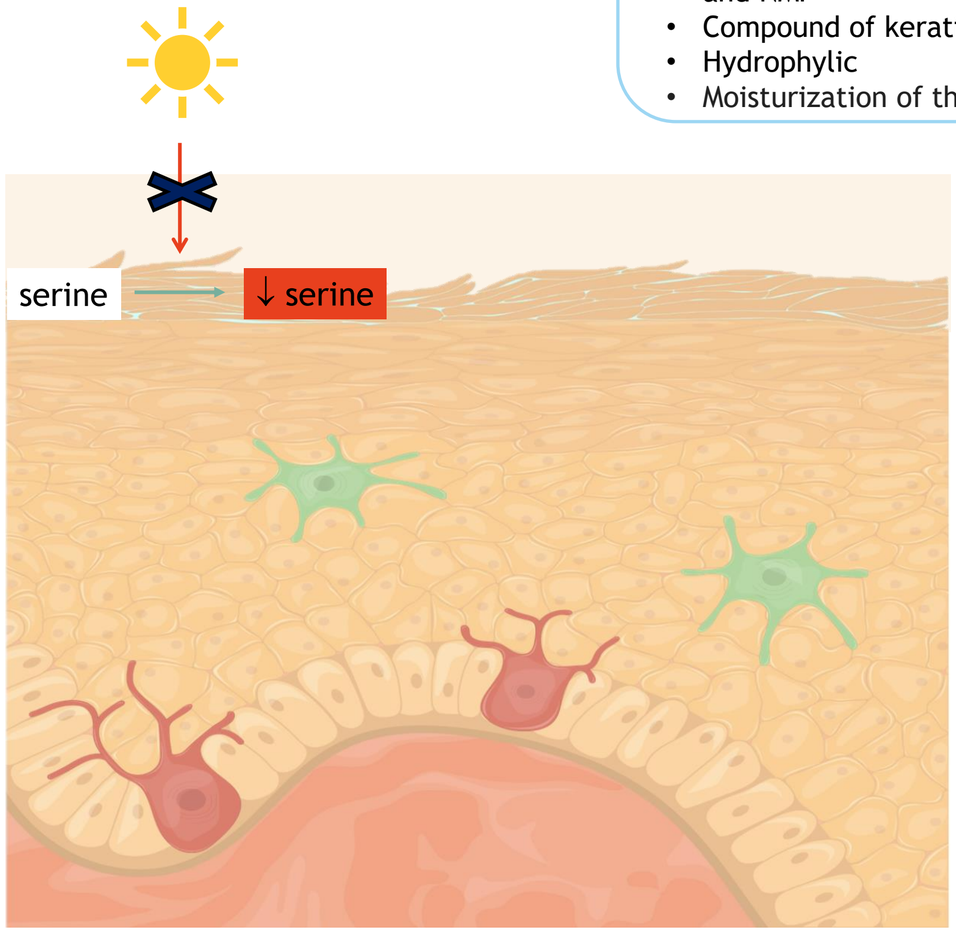
Serine



- Non-essential amino-acid
- Major component of filaggrin and NMF
- Compound of keratine
- Hydrophylic
- Moisturization of the skin



Wilcoxon test (n=19)
*p< 0.05, **p< 0.01, ***p< 0.001; ns, not significant

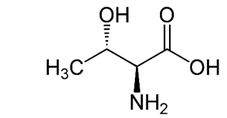


Inhibition by the products

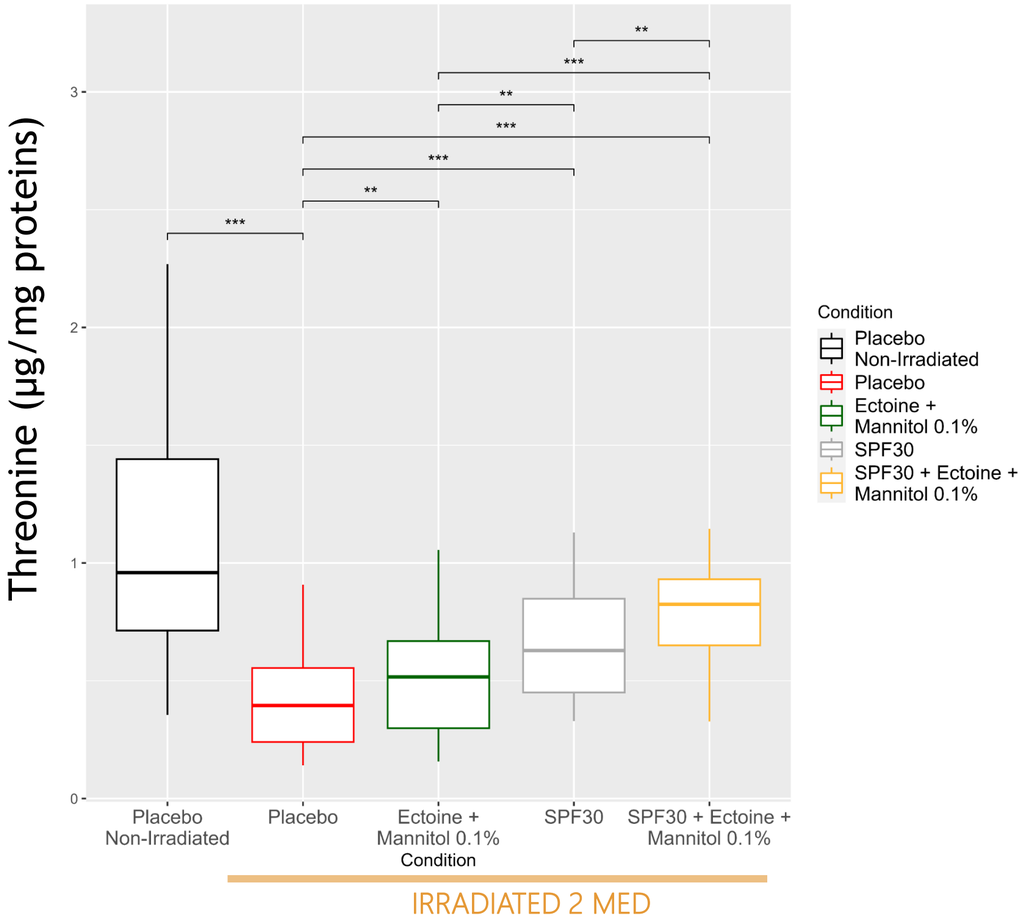
- This sunscreen product prevents serine reduction with complementary effect of filters and active ingredients

UV irradiation reduces threonine content *in vivo*

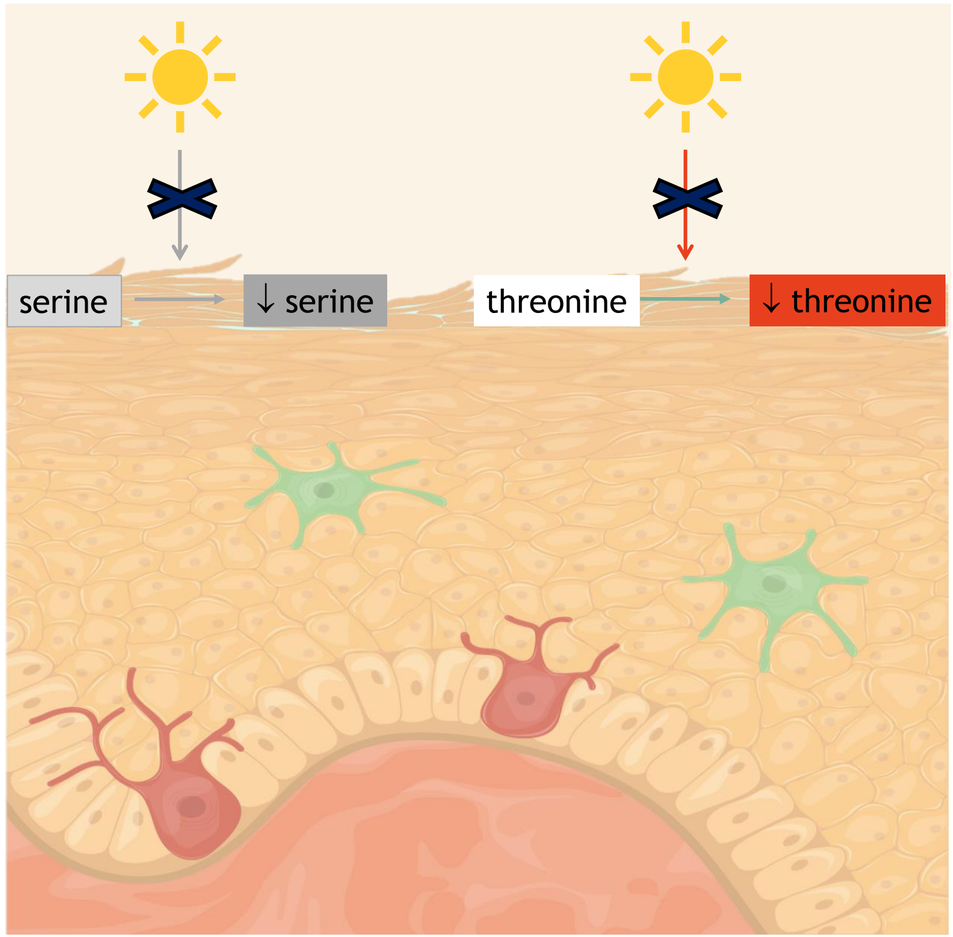
Barrier function



Threonine
- Essential amino-acid
- Hydrophylic
- Moisturization of the skin



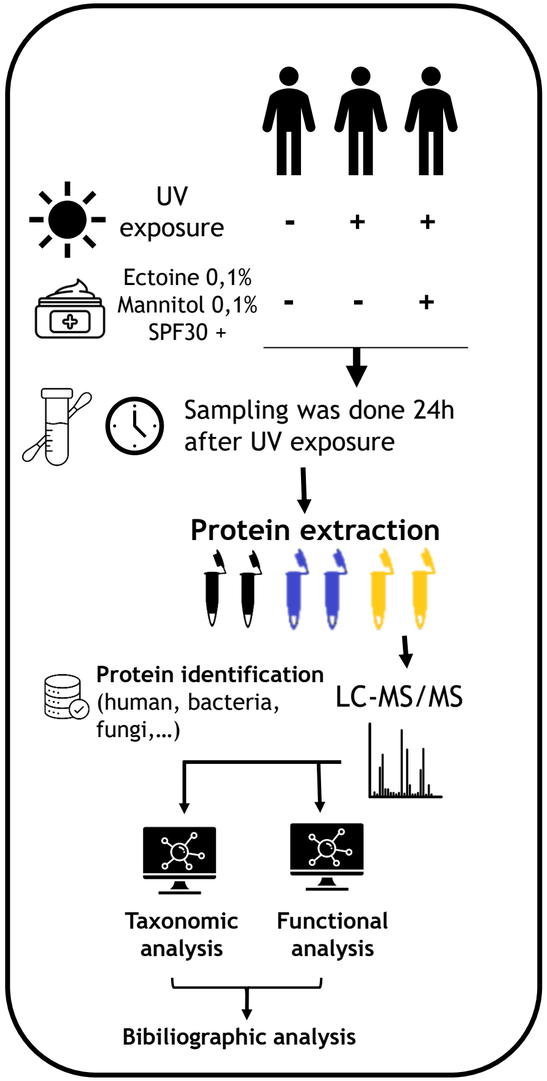
Wilcoxon test (n=19)
*p< 0.05, **p< 0.01, ***p< 0.001; ns, not significant



Inhibition by the products

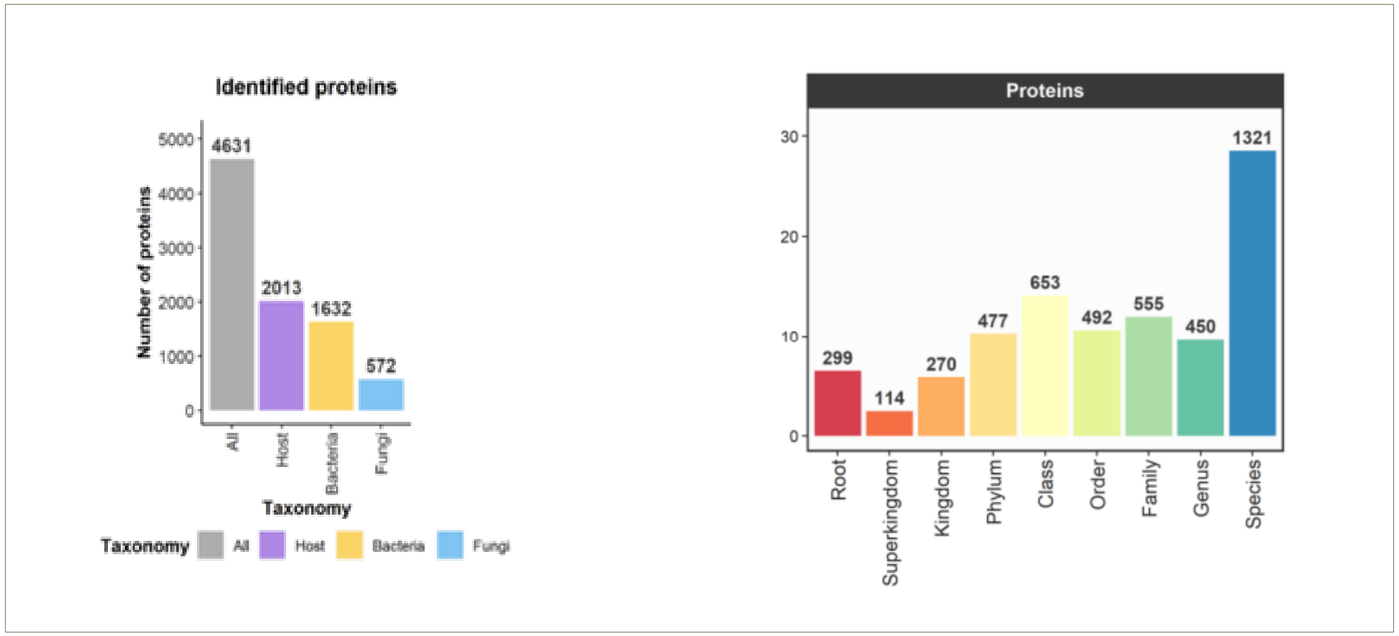
- This sunscreen product prevents serine reduction with complementary effect of filters and active ingredients

UV irradiation modifies skin metaproteome *(Preliminary data)*



Microbiome

Taxonomical distribution of identified proteins



- **4631 proteins** identified divided in 3 taxonomical classes :
  - Human
  - Bacteria
  - Fungi
- **1321 proteins** identified at the species level

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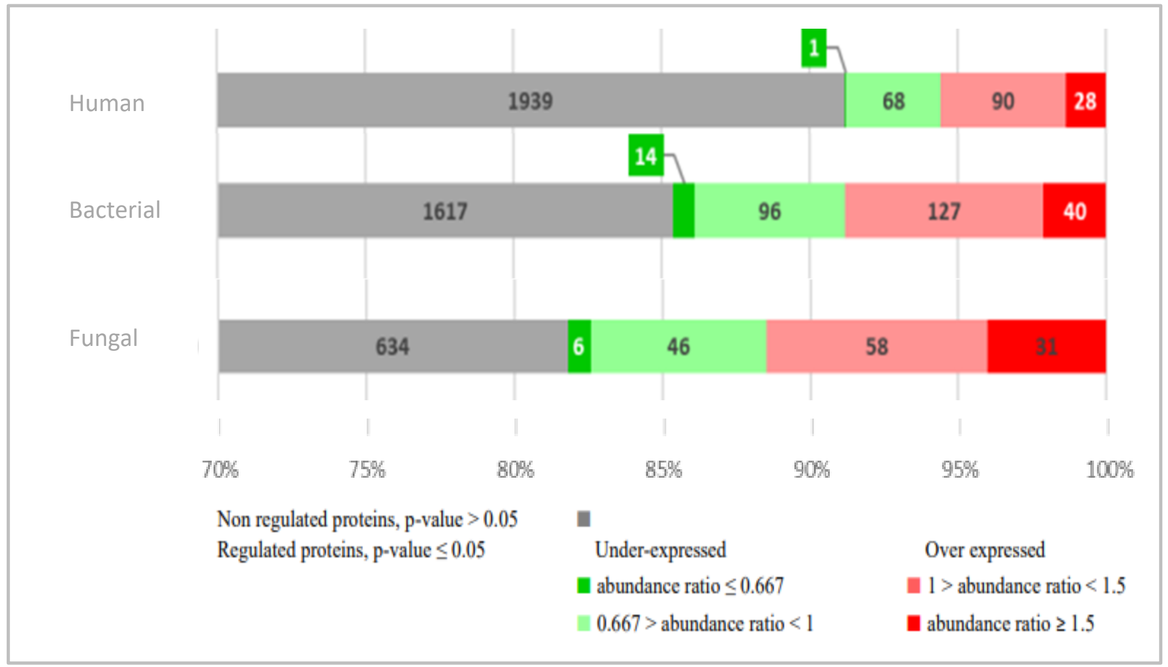
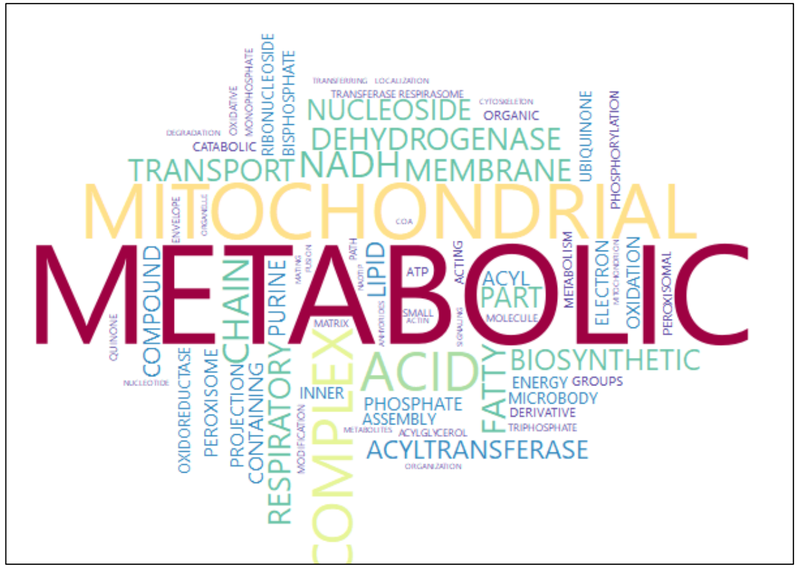
UV irradiation modifies metaproteome *(Preliminary data)*

Microbiome

Proteins distribution according to their p-values and fold changes for each taxa

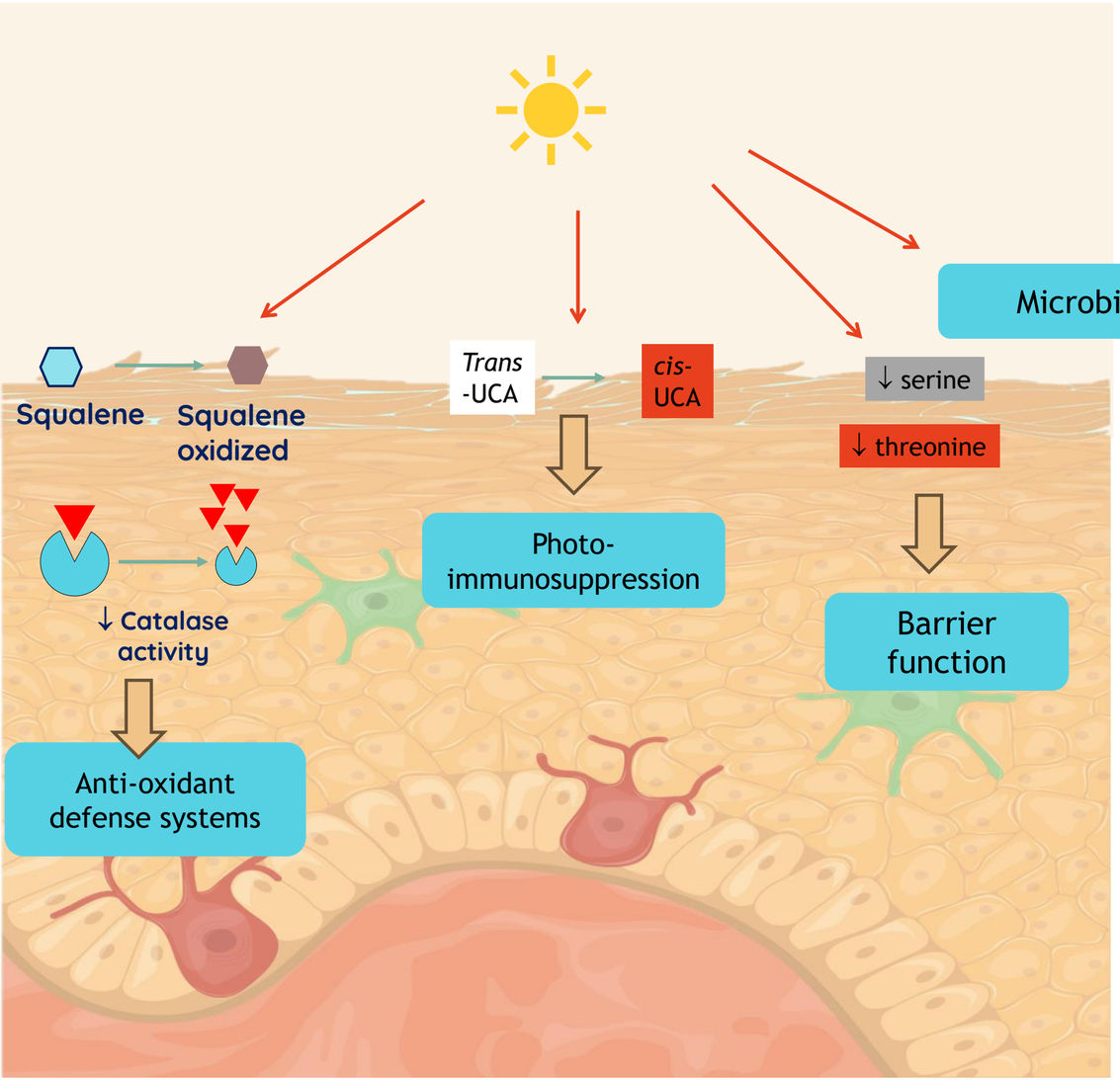


Word cloud of the most regulated pathways after UV



- Identification of several proteins modified by UV
- Activation of metabolics and energetics pathways and DNA repair proteins at human and bacterial level

Conclusions and perspectives



Conclusion

- Validation of non invasive biomarkers modulated by UV in human skin *in vivo*
- Potential tools to better understand photobiology and efficacy of sunscreen products in particular to long term effect
- Complementary analysis to regulatory tests

Perspectives…

- Find others pertinent non invasive biomarkers
- Better understand impact of UV on human skin microbiome
- Find specific microbiome biomarkers to analyse the effect of UV

Final goal : develop better suncare products with holistic benefits for the skin ecoystem under UV

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