

Analysis of Stratum Corneum lipids by HPTLC

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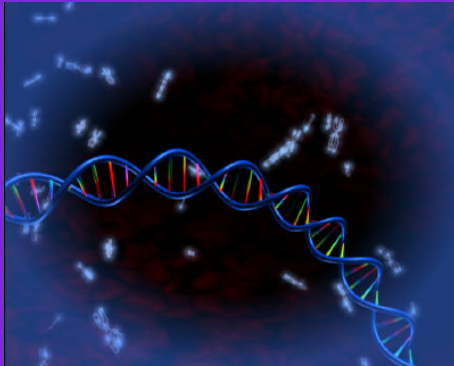
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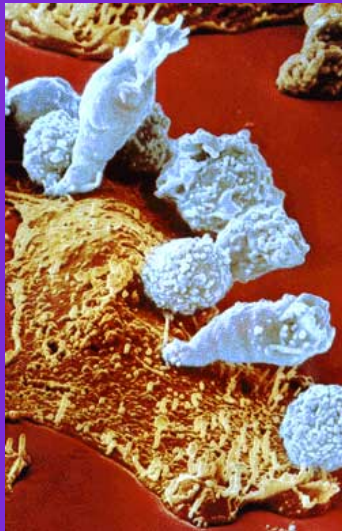
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Dermatite atopique

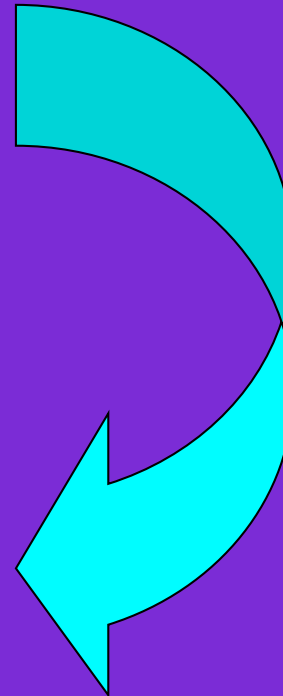
Plusieurs facteurs sont liés à cette maladie:



- génétiques
- environnementaux



- biochimiques
- immunologiques

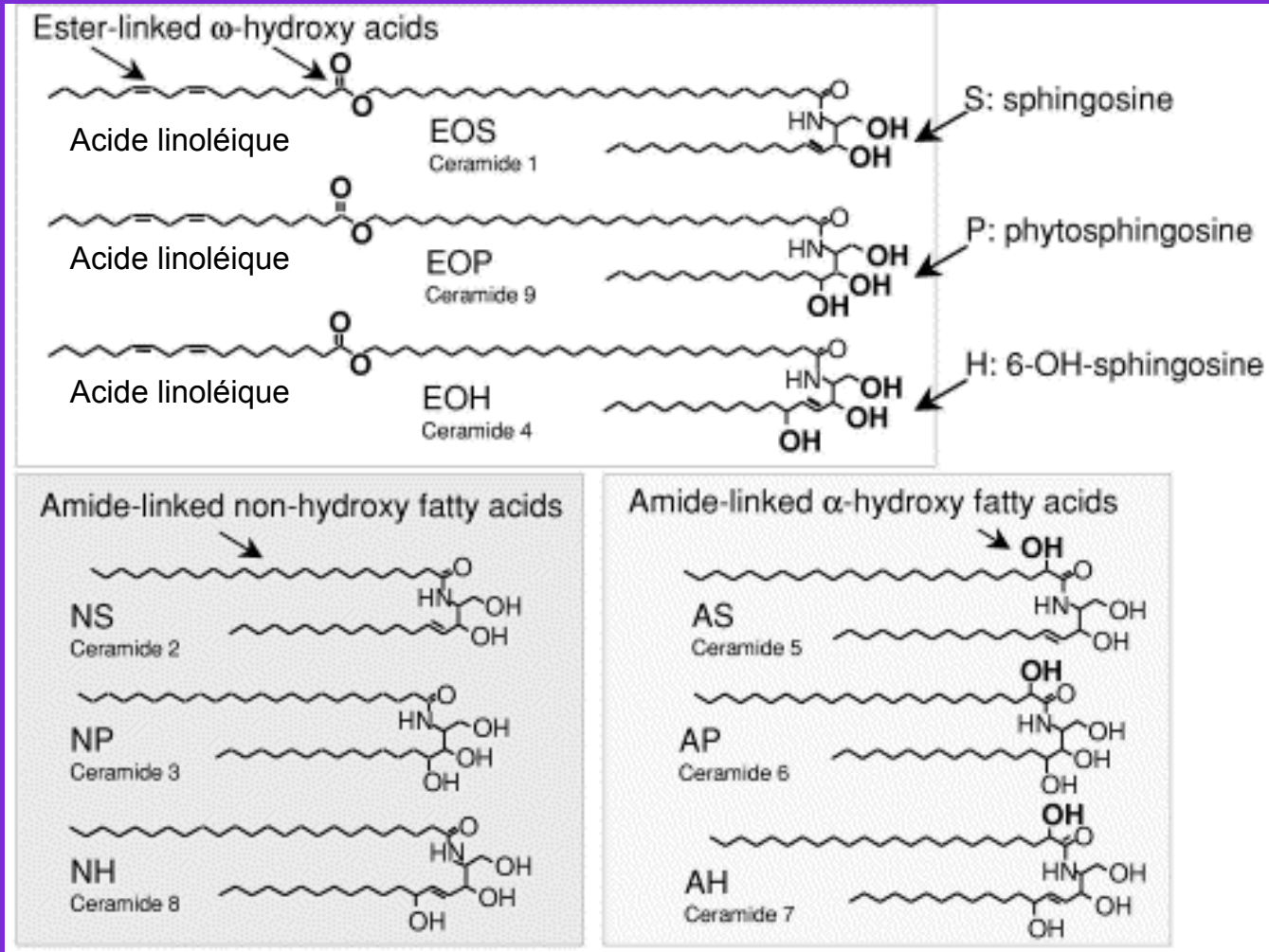


**Le chien atopique :
un bon modèle d'études
pour la dermatite
atopique humaine ?**

- correspondance clinique
- apparition spontanée chez le chien

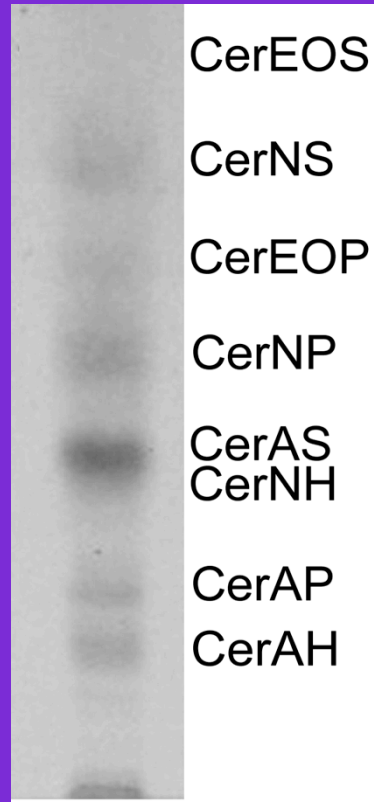


Céramides du stratum corneum humain



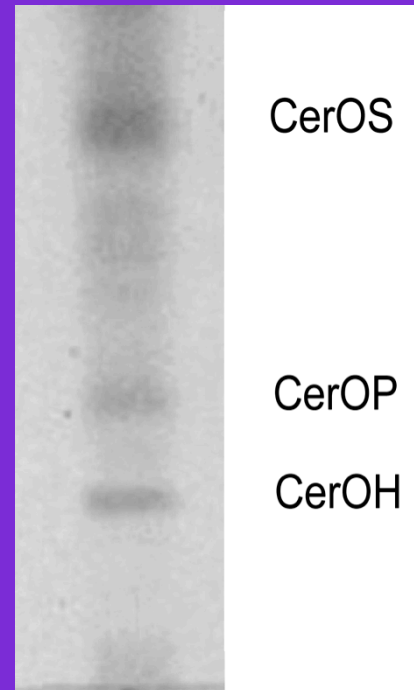
Répartition des céramides libres dans le SC humain

Cer 1 [EOS]	8%	CerEOS
Cer 2 [NS]	20%	CerNS
Cer 3 [NP]	18%	CerEOP
Cer 4 [EOH]	4%	CerNP
Cer 5 [AS]	17%	CerAS
Cer 6 [AP]	9%	CerNH
Cer 7 [AH]	12%	CerAP
Cer 8 [NH]	9%	CerAH
Cer 9 [EOP]	2%	



Répartition des céramides couplés aux protéines dans le SC humain

Cer [OS]	62%
Cer [OP]	8%
Cer [OH]	30%



Composition en céramides libres et couplés aux protéines dans le Stratum Corneum du chien et de l'homme.

	Type de céramide	Chien	Homme
Céramides libres	Cer 1 [EOS]	3%	8%
	Cer 2 [NS]	19%	20%
	Cer 3 [NP]	28%	18%
	Cer 4 [EOH]	traces	4%
	Cer 5 [AS]	33%	17%
	Cer 6 [AP]	10%	9%
	Cer 7 [AH]	6%	12%
	Cer 8 [NH]	traces	9%
	Cer 9 [EOP]	4%	2%
Céramides couplés aux protéines	Cer [OS]	62%	59%
	Cer [OP]	8%	33%
	Cer [OH]	30%	8%

Investigation of atopic dermatitis (AD) and non-atopic canine epidermal lipids modification of expression, and organization by means of:

➤ Tape Stripping.

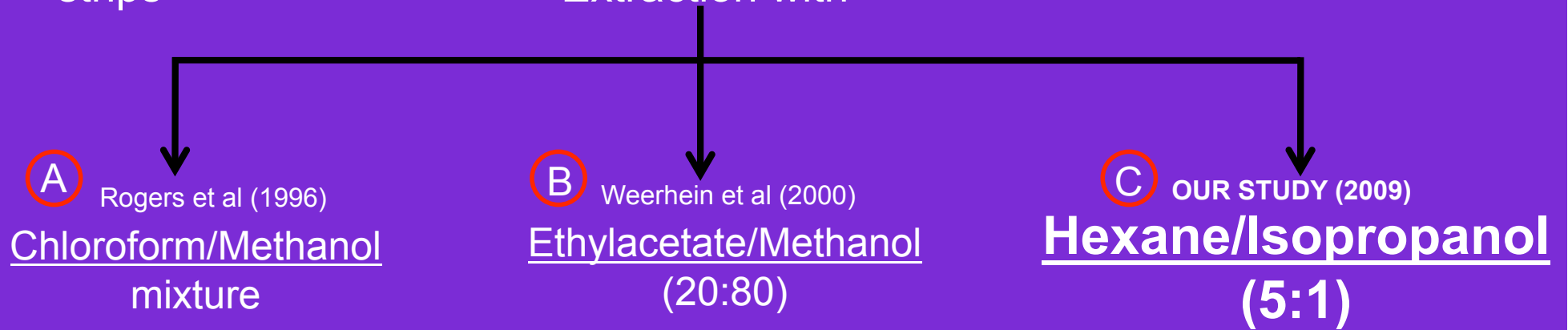
➤ High Performance Thin-Layer Chromatography (HPTLC).

Scotch Magic[®]



Methods for Skin Lipids Extraction:

1. Collection of upper Stratum Corneum (SC) by several consecutive tape strips
Extraction with



2. Collection of six consecutive scrappings followed by extraction with chloroform/methanol (*Lavrijsen et al. 1995*)

3. Collection of SC by stripping with cyanoacrylate (*Bleck et al. 1999*)

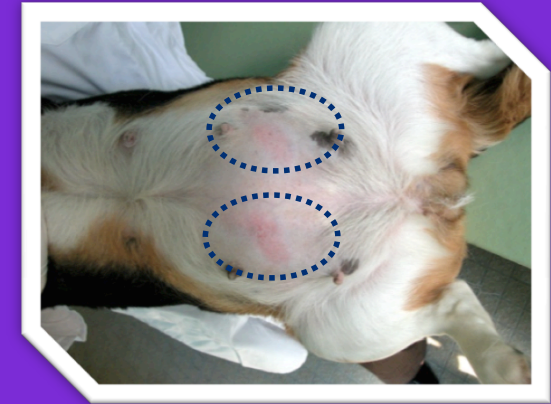
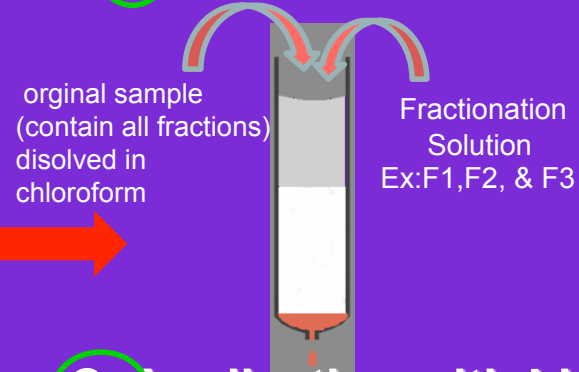


Tape stripping in combination with HPTLC to assess SC lipid:

A Extraction



B Fractionation



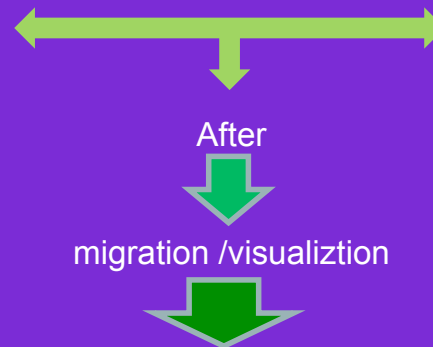
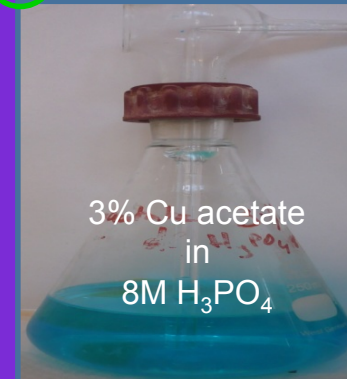
C Application with Linomat IV



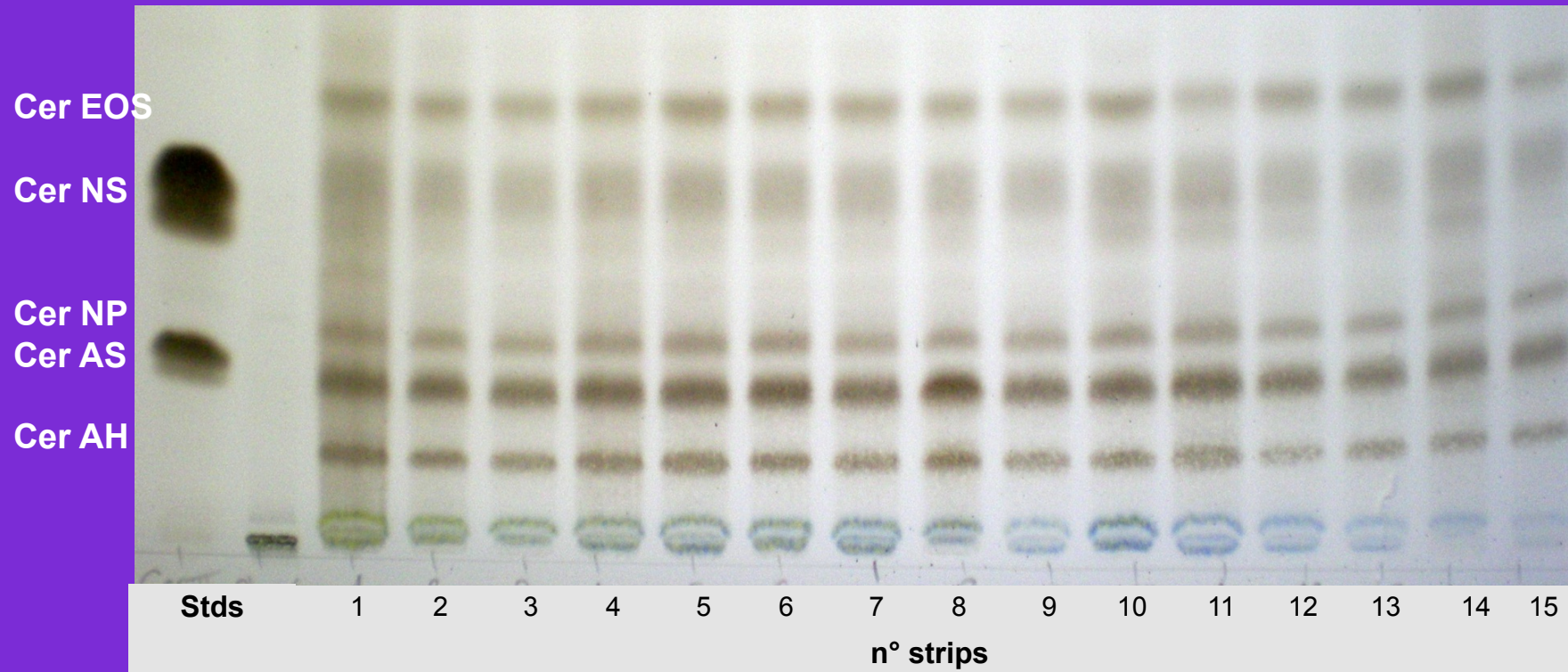
D Migration



E Visualization



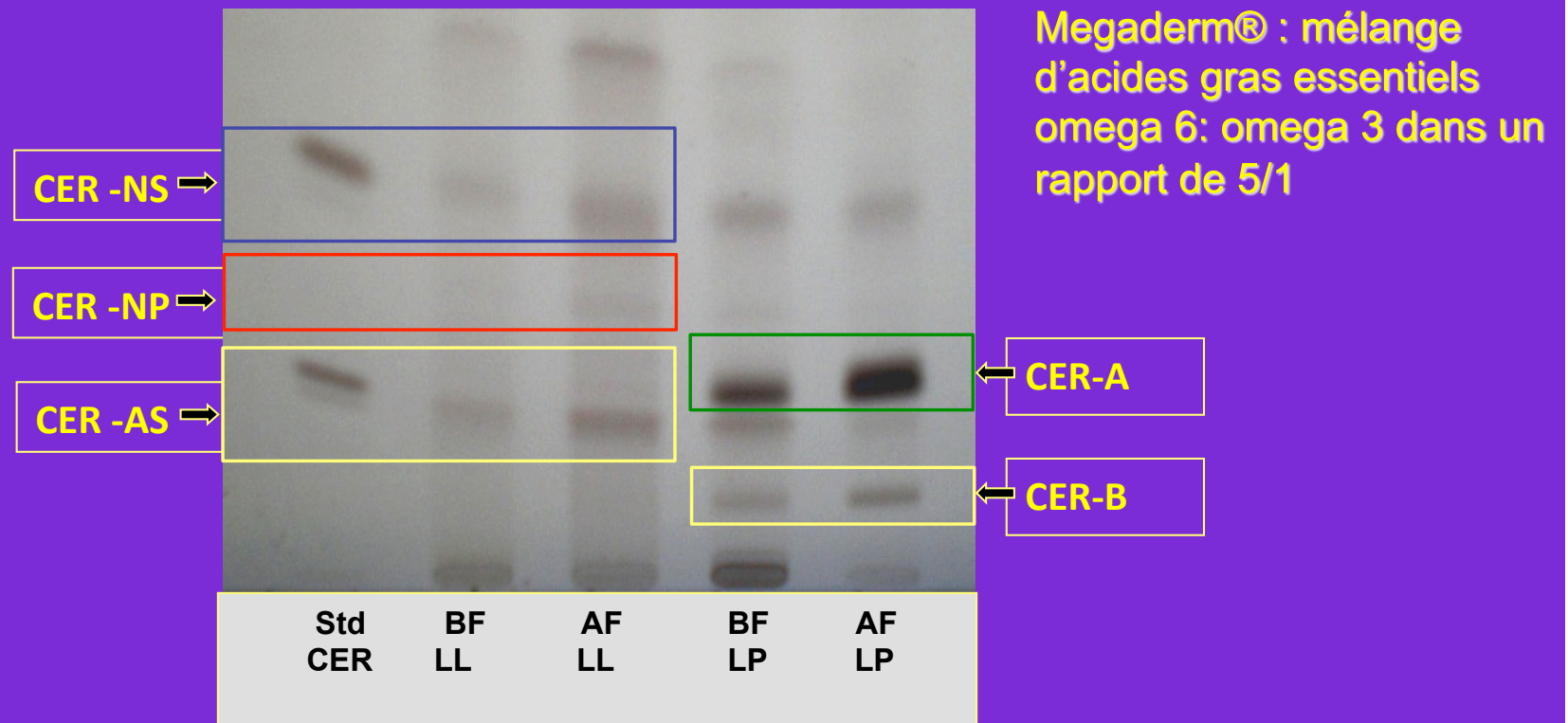
Free ceramides of healthy human Stratum Corneum



Conclusion:

The homogeneity of the stratum corneum is observed on healthy human skin
The uptake of consecutive strips shows a homogeneous distribution consistent with an efficient barrier function .

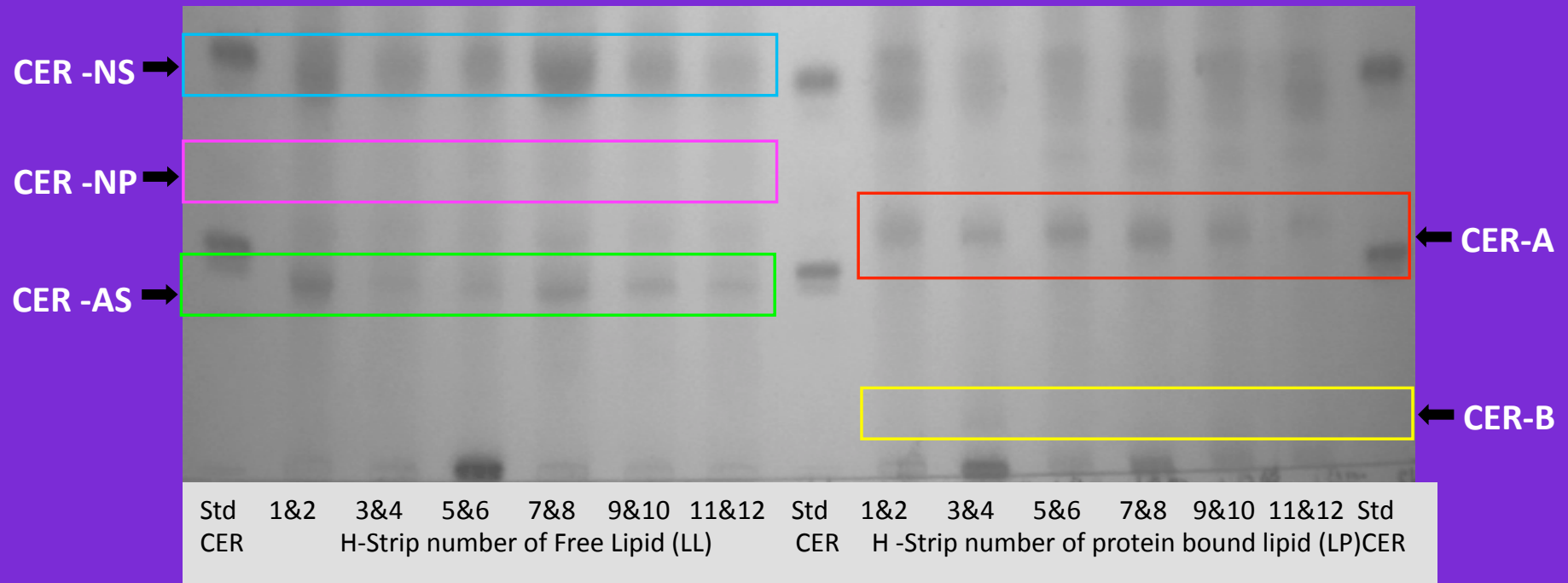
Céramides libres (LL) et liés aux protéines (LP) du chien atopique avant (BF) et après (AF) traitement oral avec Megaderm®



Augmentation globale de la quantité de céramides libres et liés
après Megaderm® → meilleure barrière cutanée

➤ Healthy Dog (2 by 2 strips)

Free Ceramides and Protein-bound ceramides



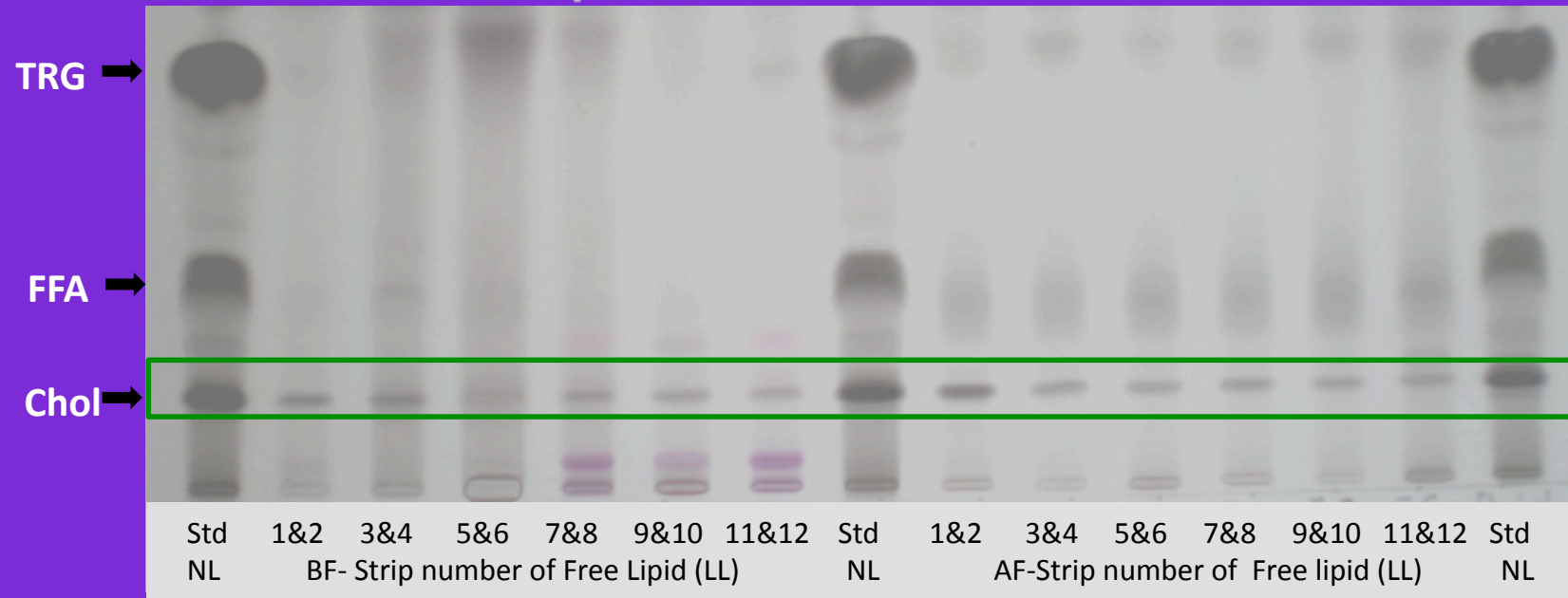
Std-CER: Standard Ceramide ;
 CER-NS: Ceramide2 (normalFA- sphingosine);
 CER-NP: Ceramides 3 (normalFA- phytosphingosine);
 CER-AS: Ceramide5 (α hydroxyFA- sphingosine);

CER-A: covalently bound ceramides Cer (OS) sphingosine- ω -hydroxy F.A;
 CER-B: covalently bound ceramides Cer (OH) 6hydroxy sphingosine- ω -hydroxy F.A.

Conclusion: heterogeneous distribution of ceramides in the strips

➤ Atopic Dog (Strips as 2 by 2)

Neutral Lipids before and after treatment



Std-LN: Neutral Lipid (5mg/ul);
Chol: Cholesterol;
BF: before treatment;

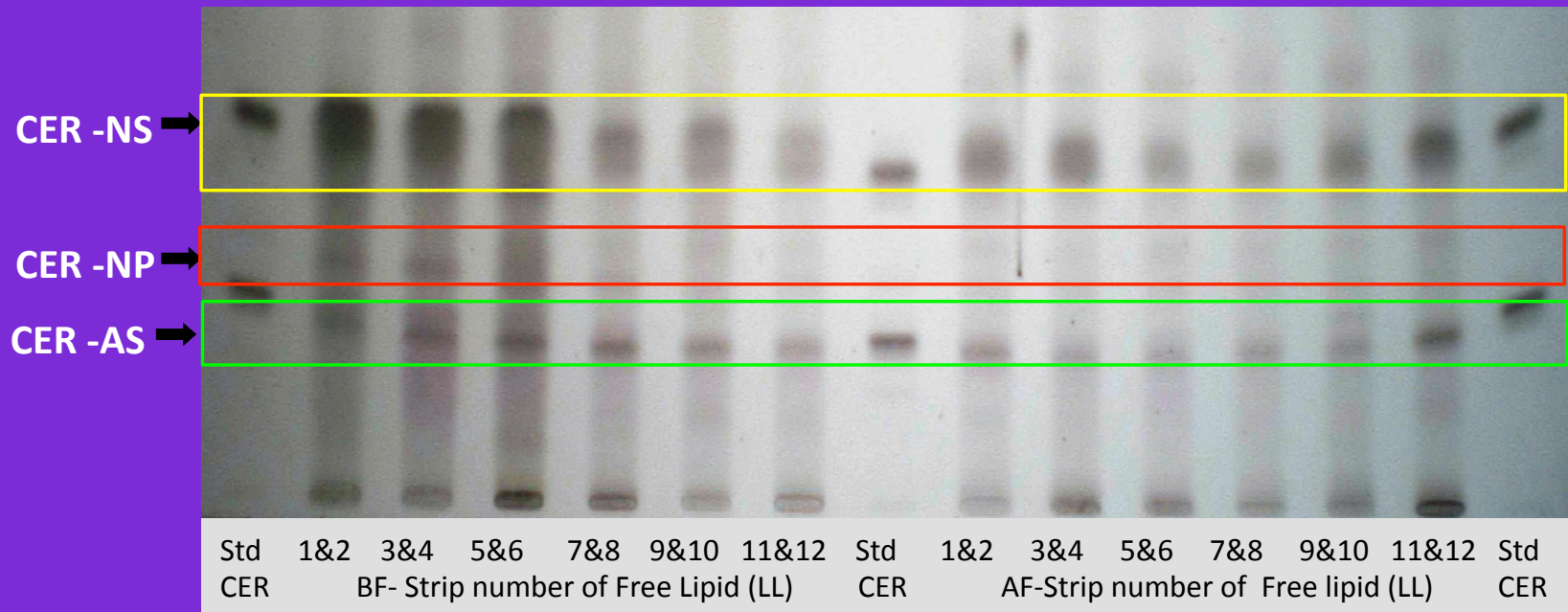
TRG: Triglyceride;
FFA: Free fatty acid;
AF: after treatment.

Conclusion:

- Homogenous elevation in bands expression after treatment with Megaderm®

➤ Atopic Dog (Strips as 2 by 2)

Free Ceramides of SC before and after treatment



Std-CER: Standard Ceramide (0.25mg/ml);
 CER-NP: Ceramides 3 (normal phytosphingosine);
 BF: before treatment ;

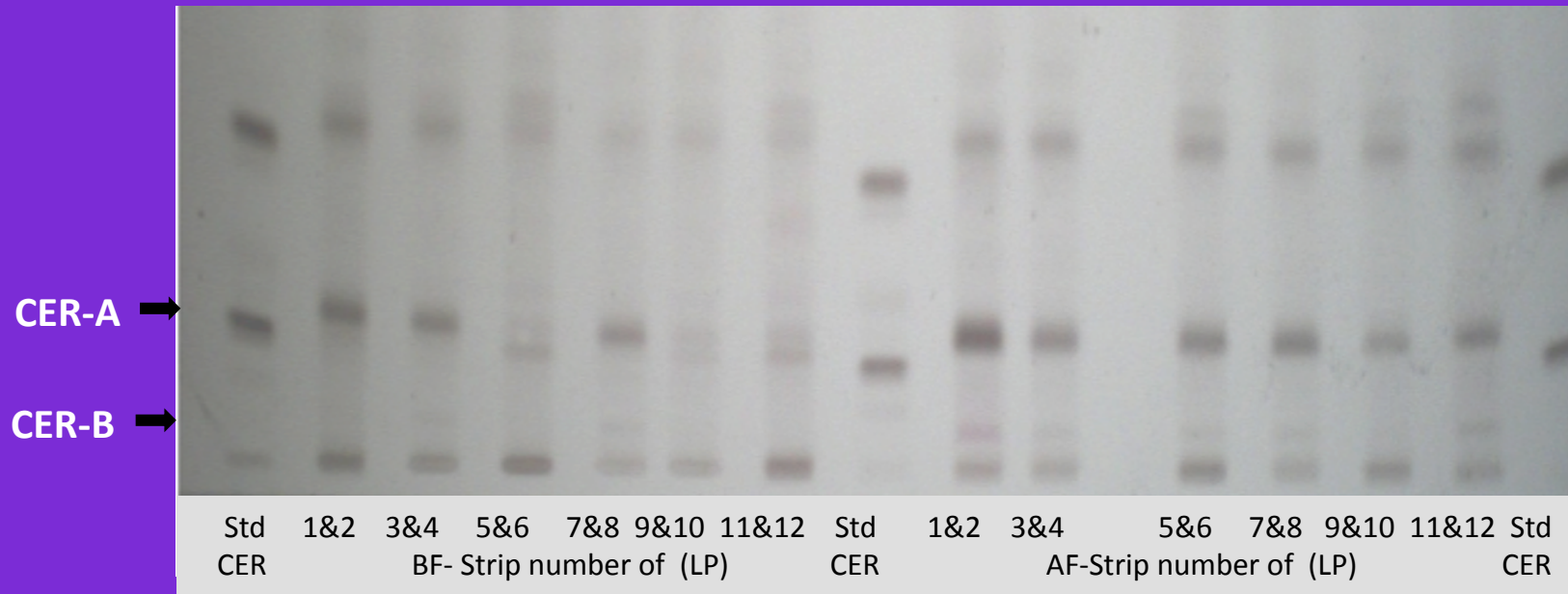
CER-NS: Ceramide2 (normal sphingosine);
 CER-AS: Ceramide5 (α hydroxy sphingosine);
 AF: after treatment.

Conclusion:

- Peculiar distribution in bands expression before treatment.
- Elevation in band expression in the deepest layer after treatment.

➤ Atopic Dog (Strips as 2 by 2)

Protein bound Ceramides before and after treatment



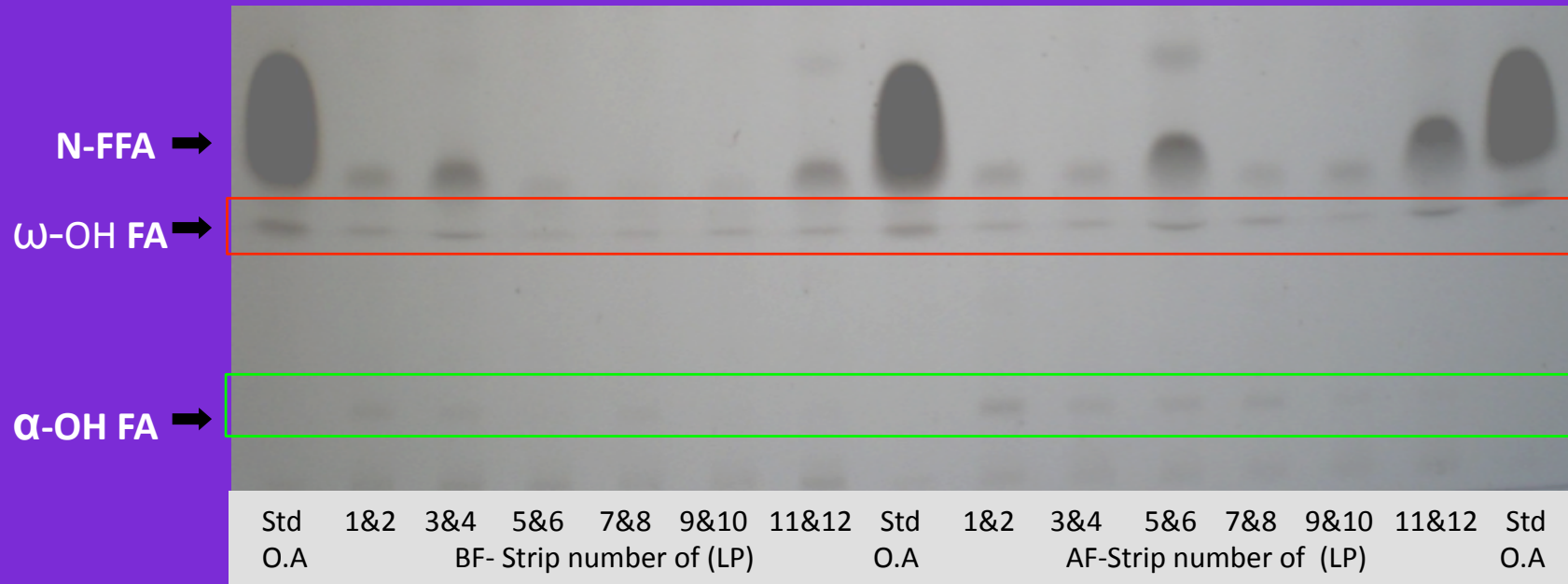
Std-CER: Standard Ceramide (0.25mg/ml); BF: before treatment; AF: after treatment;
CER-A: covalently bound ceramides Cer (OS) sphingosine- ω -hydroxy F.A;
CER-B: covalently bound ceramides Cer (OH) 6hydroxy sphingosine- ω -hydroxy F.A.

Conclusion:

- Homogenous elevation in bands expression after treatment with Megaderm®

➤ Atopic Dog (Strips as 2 by 2)

Fatty acids of SC before and after treatment



O.A: Oleic acid standard
 α-OH FA: Alpha hydroxy fatty acid;
 ω-OH FA: Omega hydroxy fatty acid.

BF: before treatment
 AF: after treatment

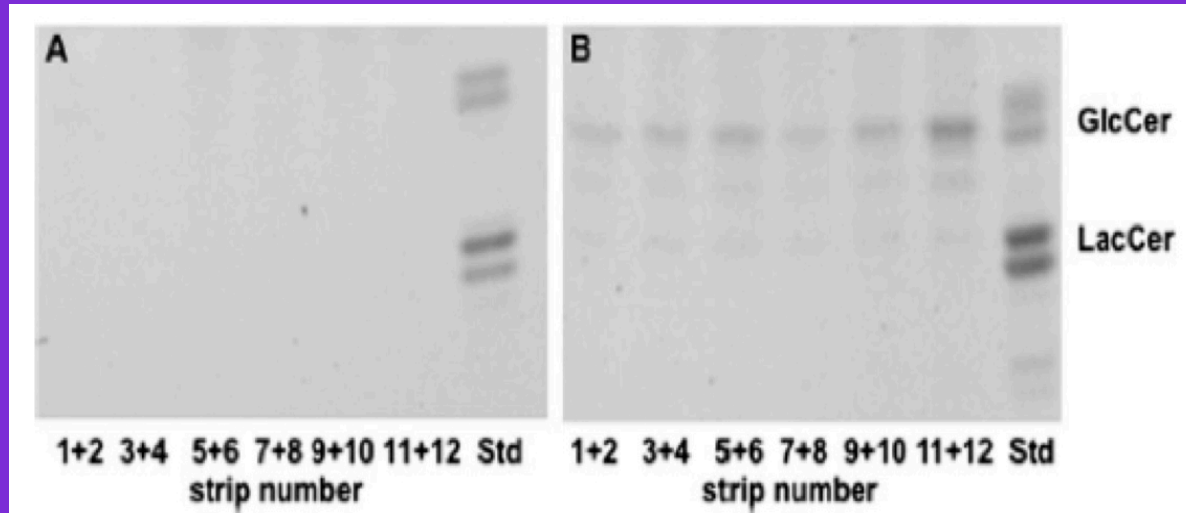
Conclusions

Elevation in fatty acids content after treatment with Megaderm®

View of distribution of protein-bound fatty acids by strips pool 2 by 2.

Présence de glucosylcéramides et de céramides liés aux protéines dans les strips successifs du SC de chien

Glucosylcéramides



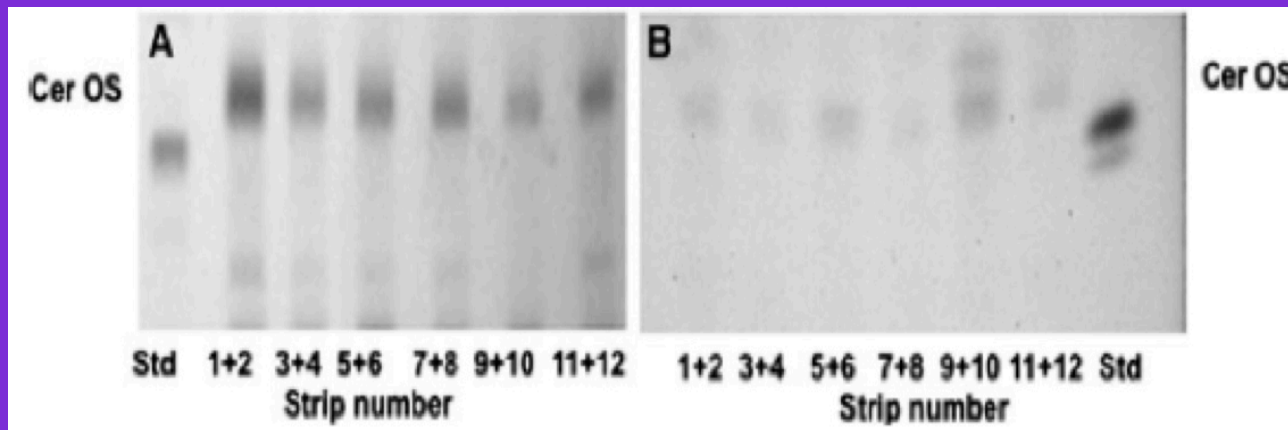
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Chien sain

Chien atopique

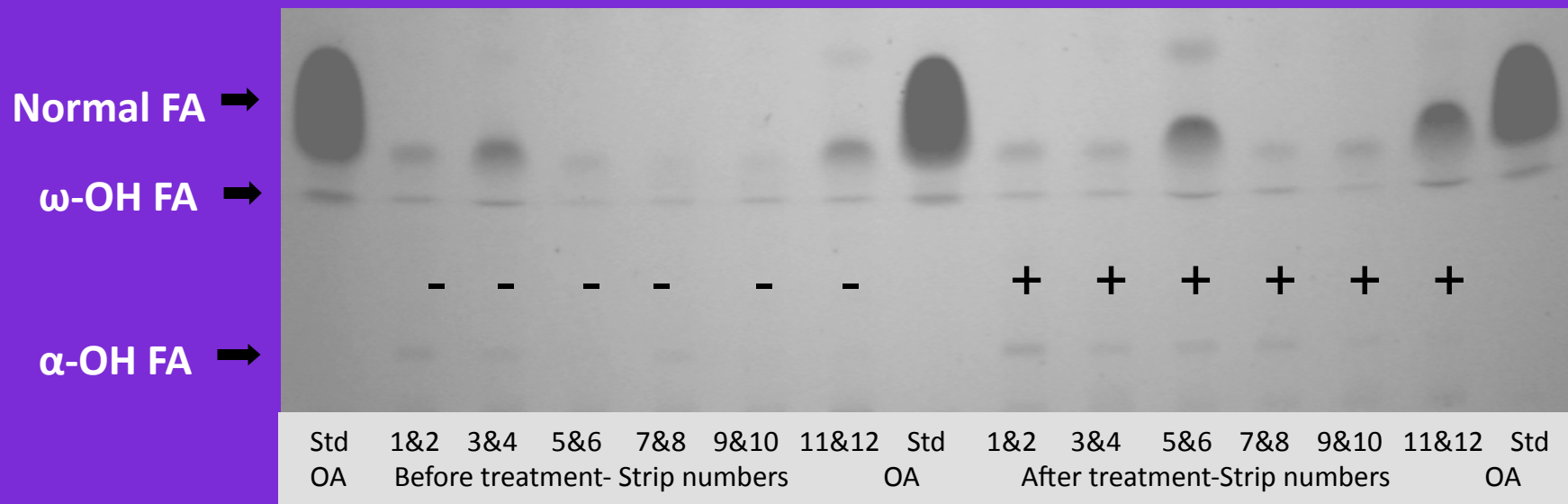
Céramides



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Acides gras liés aux protéines avant (BF) et après (AF) traitement topique avec SLC® du SC de chien atopique

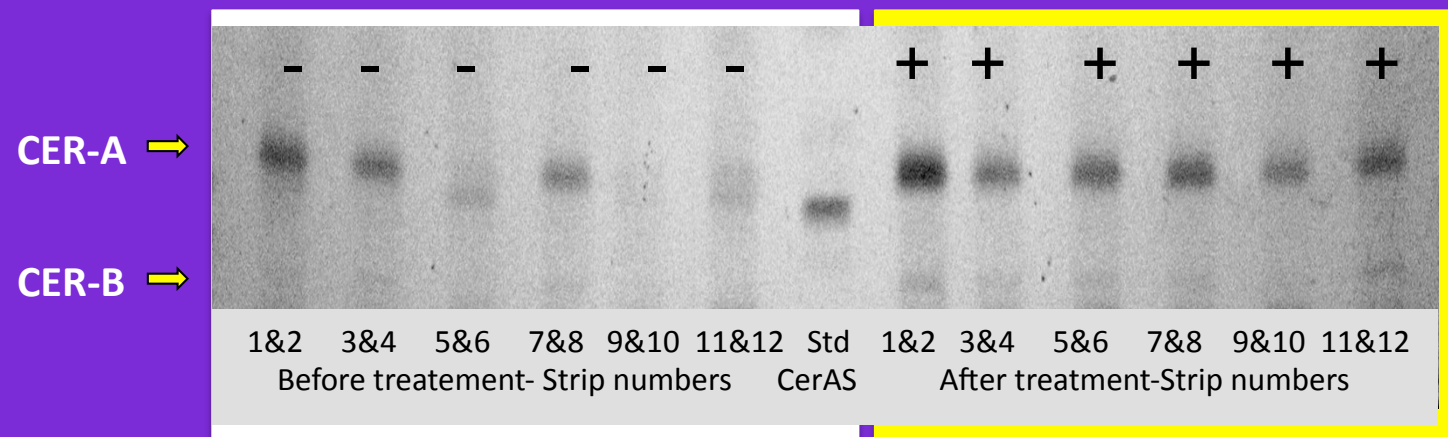


OA : Acide oléique standard;
 α-OH FA: Acides gras alpha hydroxylés
 ω-OH FA: Acides gras oméga-hydroxylés

Augmentation des acides gras alpha et oméga hydroxylés après traitement avec SLC

Céramides liés aux protéines dans le SC avant (BF) et après (AF) traitement topique avec SLC®

SLC® : émulsion à base de céramides, cholestérol et acides gras libres



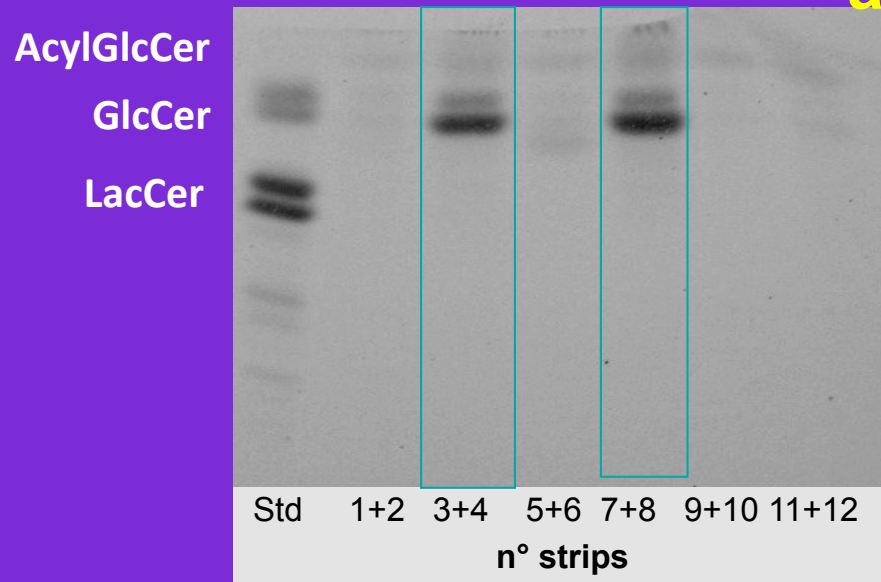
Std CerAS: Standard ceramide;

CER-A: covalently bound ceramides Cer-OS (sphingosine- ω -hydroxy FA)

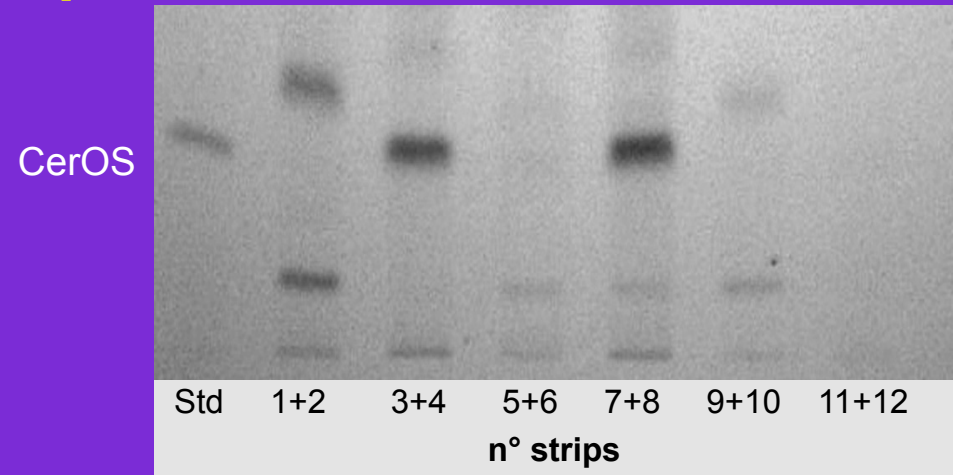
CER-B: covalently bound ceramides Cer-OH (6hydroxy sphingosine- ω -hydroxy FA)

Après traitement, la répartition des céramides dans les couches successives redevient homogène et la quantité globale augmente

Répartition des sphingolipides liés aux protéines dans le stratum corneum de patients souffrant de dermatite atopique



Glucosylcéramides



Céramides

- Les glucosylcéramides qui sont normalement absents du stratum corneum se répartissent de manière hétérogène dans les couches successives.
- Les céramides à acides gras oméga-hydroxylés sont aussi répartis de manière hétérogène

L'important est d'analyser la composition lipidique couche par couche