

# Hexamidine, a Protease Inhibitor, Promotes Stratum Corneum Lipid Biomarkers *In Vitro*

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## INTRODUCTION

Skin aging has been described as a cumulative process resulting from unrepaired damage as well as age-related physiological changes. The damaging factors which accelerate skin aging can be divided into intrinsic factors, such as free radicals, and extrinsic factors, with UV exposure being the most important. Within the current study we examined the effects of both intrinsic aging and photoaging on the expression of stratum corneum (SC) lipid metabolism pathways. Human skin equivalent (SE) cultures were used to monitor these pathways in response to hexamidine, a protease inhibitor.

## OBJECTIVE

The goal of the current work was to evaluate the effects of intrinsic aging and photoaging on gene expression of SC lipid metabolism pathways *in vivo* and monitor these pathways *in vitro* in response to hexamidine.

## BACKGROUND

The major lipids of the human SC are ceramides, cholesterol, and fatty acids, comprising approximately 50%, 25%, and 10% of the total lipid mass, respectively<sup>1</sup>. These mature SC lipids are generated from precursor lipids<sup>2,3</sup> which are synthesized, packaged into lamellar bodies (LB), and then released into the SC extracellular space following LB fusion with the plasma membrane of granular keratinocytes<sup>4</sup>. Subsequent lipid processing yields the mature SC lipids [Figure 1]<sup>2,3</sup>.

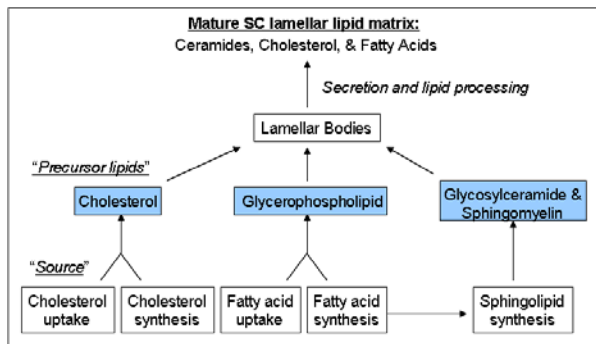


Figure 1. Summary of the *in vivo* pathways to form mature SC lamellar lipids.

## METHODS

RNA was purified from full thickness skin biopsies from individual study subjects [young (18-20 yrs of age) or older (60-67 yrs of age) females] buttocks (UV protected) and outer forearm (UV exposed). RNA was labeled and hybridized to Affymetrix Gene Chips. Following statistical analysis, bioinformatics focused on expression of genes specific to metabolism of SC lipids. SEs treated topically with 0.1% hexamidine (Figure 2) for 32hrs were processed for gene chip analysis as stated above.

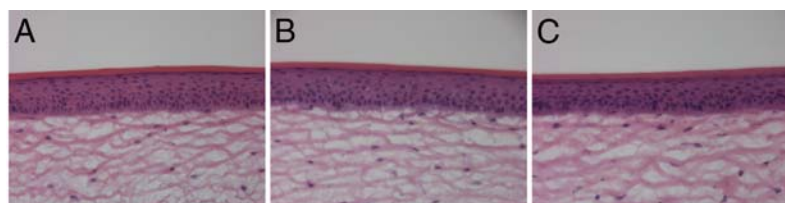


Figure 2. Representative images illustrating the histological appearance of SE cultures, (A) non-treated, (B) vehicle treated for 32hrs, and (C) hexamidine treated for 32hrs.

## SUMMARY

In both intrinsic aging and photoaging there was a coordinated down-regulation of genes involved in SC lipid metabolism pathways. In contrast, treatment of SE cultures with 0.1% hexamidine led to increased expression of lipid metabolism genes as biomarkers of an improved skin barrier.

- As compared to younger skin, the expression of genes involved in fatty acid production were down-regulated in both intrinsic aging and photoaging. In contrast, treatment of SE with hexamidine led to increased expression of these genes as biomarkers of an improved skin barrier [Table I].

- Similarly, the expression of cholesterol synthetic pathway genes were down-regulated in both intrinsic aging and photoaging. Treatment of SE with hexamidine led to increased expression of these genes as biomarkers of an improved skin barrier [Table II].

- Sphingolipid biosynthesis and processing were also down-regulated, however this was more pronounced in intrinsically aged than photoaged skin. Treatment of SE with hexamidine led to a moderate increase in expression of these barrier biomarkers [Table III].

## CONCLUSIONS

- The coordinated down-regulation of SC lipid pathways likely contributes to the decreased capacity of aged skin to maintain and repair the SC barrier.
- The SE data suggest this state can be improved by hexamidine and therefore may be a good candidate for cosmetic applications.

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References  
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 2. Mao-Qiang M, Feingold KR, Jain M, Elias PM. Extracellular processing of phospholipids is required for permeability barrier homeostasis. J Lipid Res. 1995 Sep;36(9):1925-35.  
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## RESULTS

Figures 3, 4 and 5 highlight the biochemical pathways by which SC cholesterol, fatty acids and ceramides are produced. The key intermediates are given in yellow, while the enzymes responsible for their formation are given in red. Tables I, II, and III highlight the expression of these genes in intrinsic aging, photoaging and SE topically treated with 0.1% hexamidine for 32hrs.

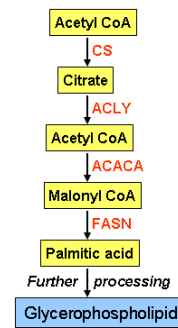


Figure 3. Pathway for the production and metabolism of fatty acids.

Table I. Expression of genes responsible for fatty acid formation. Red= up-regulation (p≤0.05). Blue=down-regulation (p≤0.05).

Acronym	Gene Name	Fold Change Older vs. Younger		Fold Change Veh vs. Treated
		Intrinsic aging (Buttock samples)	Photoaging (Forearm samples)	SE treated with 0.1% hexamidine
CS	Citrate synthase	-1.2	-1.23	
ACL	ATP citrate lyase	-1.57	-1.44	1.69
ACACA	Acetyl-Coenzyme A carboxylase alpha	-1.32	-1.52	1.68
FASN	Fatty acid synthase	-2.37	-2.26	1.57

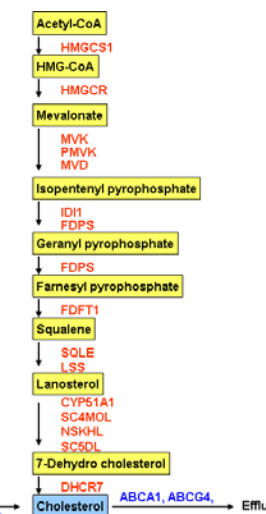


Figure 4. Pathway for the production and metabolism of cholesterol.

Table II. Expression of genes involved in cholesterol production. Red= up-regulation (p≤0.05). Blue=down-regulation (p≤0.05).

Acronym	Gene Name	Fold Change Older vs. Younger		Fold Change Veh vs. Treated
		Intrinsic aging (Buttock samples)	Photoaging (Forearm samples)	SE treated with 0.1% hexamidine
<b>Cholesterol Biosynthetic Genes</b>				
HMGCS1	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1	-1.87	-1.8	1.83
HMGR	3-hydroxy-3-methylglutaryl-Coenzyme A reductase	-1.32	-1.32	1.36
MVK	Mevalonate kinase	-1.57	-1.53	
PMVK	Phosphomevalonate kinase	-1.47	-1.69	
MVD	Mevalonate decarboxylase	-1.72	-2.18	
IDI1	Isopentenyl-diphosphate delta isomerase 1	-1.36	-1.24	2.22
FDPS	Farnesyl diphosphate synthase	-1.41	-1.67	1.47
FDDT1	Farnesyl-diphosphate farnesyltransferase 1	-1.36	-1.37	2.04
SQLE	Squalene epoxidase	-1.39	-1.82	1.43
LSS	Lanosterol synthase		1.13	
CYP51A1	Cytochrome P450, family 51, subfamily A, polypeptide 1	-1.48		1.36
SC4MOL	Sterol-C4-methyl oxidase-like	-1.83	-1.71	1.48
NSDHL	NAD(P) dependent sterol dehydrogenase-like	-1.66	-1.71	1.33
DHCR7	7-dehydrocholesterol reductase	-1.65	-1.65	1.53
<b>Cholesterol Uptake and Efflux Genes</b>				
LDLR	Low density lipoprotein receptor	-1.54		2.96
SCARB1	Scavenger receptor class B, member 1	-1.28		-1.60
ABCA1	ATP-binding cassette, sub-family A, member 1	1.43	1.56	1.52
ABCG4	ATP-binding cassette, sub-family G, member 4			-2.21

Table III. Expression of genes responsible for sphingolipid formation. Red= up-regulation (p≤0.05). Blue=down-regulation (p≤0.05).

Acronym	Gene Name	Fold Change Older vs. Younger		Fold Change Veh vs. Treated
		Intrinsic aging (Buttock samples)	Photoaging (Forearm samples)	SE treated with 0.1% hexamidine
<b>Sphingolipid Biosynthetic and Processing Genes</b>				
SPTLC1	Serine palmitoyltransferase, long chain base subunit 1	-1.23	1.41	1.12
SPTLC2	Serine palmitoyltransferase, long chain base subunit 2	-1.35	1.11	1.94
FVT1	Follicular lymphoma variant translocation 1	1.12		1.70
LASS2	LAG1 longevity assurance homolog 2		1.37	
LASS4	LAG1 longevity assurance homolog 4	-1.51	-1.54	-1.50
LASS5	LAG1 longevity assurance homolog 5			
LASS6	LAG1 longevity assurance homolog 6		-1.44	-1.60
DEGS1	Degenerative spermatocyte homolog 1, lipid desaturase		-1.32	-1.70
DEGS2	Degenerative spermatocyte homolog 2, lipid desaturase	-1.69	-1.52	-1.50
UGCG	UDP-glucose ceramide glucosyltransferase	-1.23	-1.94	1.45
MGC26963	Hypothetical protein MGC26963			
TMEM23	Transmembrane protein 23		1.31	1.76
GBA	Glucosidase, beta; acid	-1.32	-1.17	
SMPD1	Sphingomyelin phosphodiesterase 1			1.42
SMPD2	Sphingomyelin phosphodiesterase 2			

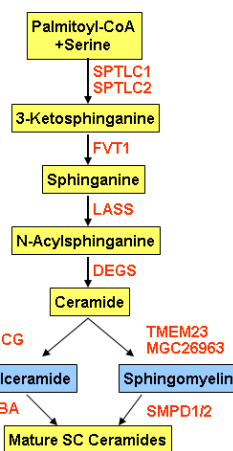


Figure 5. Pathway for the production and metabolism of sphingolipids.