Lipid mediator class-switching defects in aging skin disrupt pro-resolving pathways and compromise dermal homeostasis

Amandine Lopez-Gaydon[1], Adeline Rascalou[1], Sébastien Bonnet[1], Emeline Van-Goethem[2], Gérald Chêne[2], Nicolas Bechetoille[1]

[1] Gattefossé R&D, Saint-Priest, France
[2] Ambiotis, Toulouse, France

INTRODUCTION & OBJECTIVE

Specialized pro-resolving mediators (SPMs)—including lipoxins, resolvins, protectins, and maresins—are endogenous lipid mediators derived from DHA, EPA, and arachidonic acid that actively drive inflammation resolution and restore tissue homeostasis. In the skin, these mediators are produced by both resident and infiltrating cells, with macrophages as key sources and fibroblasts expressing their receptors, thereby supporting macrophage–fibroblast crosstalk that orchestrates tissue remodeling and repair. Aging is associated with a decline in pro-resolving capacity and impaired SPM signaling, contributing to inflammaging, although the underlying molecular mechanisms in the skin remain poorly understood. In particular, it remains unclear whether age-related disruption of macrophage–fibroblast communication contributes to defective resolution processes and compromised tissue repair.

We therefore performed a comprehensive metabololipidomic analysis of 25 lipid mediators in human skin explants from young (22.7 ± 5.2 years) and elderly donors (66.7 ± 7.3 years) following PMA-induced inflammatory stress, to precisely characterize age-related alterations in SPM pathways, determine their impact on macrophage–fibroblast crosstalk, and assess the resulting consequences for inflammation resolution and skin tissue homeostasis.

MATERIALS & METHODS

Skin explants from young (18, 21, 22, and 25 years old) and elderly donors (67, 70, and 76 years old) were obtained from surgical discard tissue following informed patient consent, in accordance with the ethical guidelines of the Tissue Bank Institute of Edouard Herriot Hospital (Lyon, France). Skin explants were cultured in Snapwell™ inserts (Corning) at the air–liquid interface in Prime-3D medium (CnT-PR-3D, CELLnTEC) supplemented with Normocin™ (InvivoGen), and maintained at 37°C under 5% CO_2. Explants were topically treated with 50 µL of a gel formulation containing 1.5% phorbol 12-myristate 13-acetate (PMA; Sigma) for up to 48 h. Explants treated with the corresponding PMA-free gel formulation served as controls.

Normal human dermal fibroblasts were isolated from an abdominal skin biopsy obtained from a 19-year-old Caucasian female donor. Cells were cultured in Fibroblast Growth Medium® (FGM®, Lonza) at 37°C under 5% CO_2 and treated with lipid mixtures for 48 h at passage 8. Untreated cells served as controls.

Macrophages were differentiated from human peripheral blood mononuclear cells (PBMCs). PBMCs were isolated from buffy coats obtained from healthy blood donors using a standard Ficoll–Hypaque density gradient centrifugation method. Monocytes were subsequently isolated from PBMCs by plastic adherence for 2 h in serum-free macrophage culture medium (M-SFM, Gibco) at 37°C under 5% CO_2. Monocytes were then differentiated into $M_{M\text{-}CSF}$ macrophages (also referred to as M0 macrophages) according to a proprietary in-house protocol involving a defined growth factor supplementation schedule. Untreated cells served as controls.

Bioactive lipid quantification was performed by LC–MS/MS using an UHPLC system (ExionLC AD, SCIEX) coupled to a QTRAP 6500+ mass spectrometer (SCIEX) equipped with an electrospray ionization source operating in negative ion mode. For skin explants, whole tissues were analyzed and results were expressed as lipid content per tissue weight (µg/mg). The area under the curve (AUC) was calculated using the trapezoidal rule, and AUC values were subsequently subjected to principal component analysis (PCA) using TANAGRA software. For fibroblasts and macrophages, the entire culture content (cells and culture supernatant) was collected and analyzed. Results were expressed as lipid concentration in the culture medium (pg/mL). Lipid ratios were subsequently calculated and expressed in arbitrary units.

MMP-3 enzymatic activity and mRNA expression levels were analyzed in normal human dermal fibroblasts. Culture supernatants were collected for the assessment of enzymatic activity using the SensoLyte® Assay Kit (CliniSciences), according to the manufacturer's instructions. Total RNA was extracted using the RNeasy Mini Kit (Qiagen) following the manufacturer's protocol. mRNA was reverse-transcribed into cDNA using the PrimeScript™ RT Reagent Kit (TaKaRa Bio). Quantitative real-time PCR was performed using SYBR® Premix Ex Taq™ II (TaKaRa Bio) on an AriaMx Real-Time PCR System (Agilent). Gene expression levels were normalized to the TBP housekeeping gene, and relative expression was calculated using the 2^−ΔΔCt method.

RESULTS

Aging profoundly remodels the lipid mediator landscape in skin

Young skin displayed a pronounced activation of the arachidonic acid cascade (figure 1) with increased biosynthesis of pro-resolving mediators LXA4 and LXB4 (figure 2). In contrast, elderly skin preferentially mobilized EPA/DHA-derived pathways.

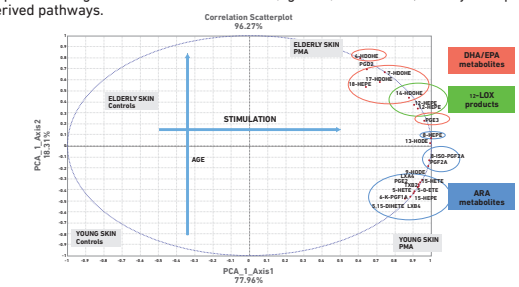


Figure 1. Comparative metabololipidomic profiling of bioactive lipids in PMA-challenged skin explants from young and elderly donors: AUC analysis followed by PCA.

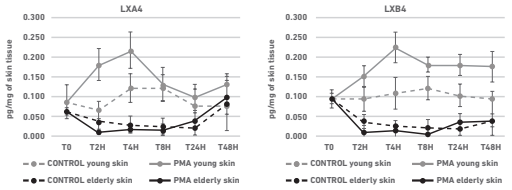


Figure 2. Time-course production of LXA4 and LXB4 in PMA-challenged skin explants from young and elderly donors.

The resolution-to-inflammation ratio—defined as (15-HETE, LXB4)/(PGE2, 6-keto-PGF1α, TXB2) and referred to as the resolution inflammatory index—was consistently higher in young donors (figure 3).

Together, these results indicate that aging drives a shift from pro-resolving to pro-inflammatory lipid signaling (figure 4).

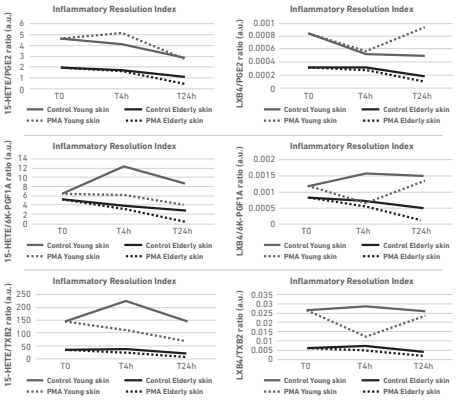


Figure 3. Bioactive lipid ratio—defined as pro-resolving mediators (15-HETE, LXB4)/pro-inflammatory mediators (PGE2, 6-keto-PGF1α, TXB2)—in PMA-challenged skin explants from young and elderly donors.

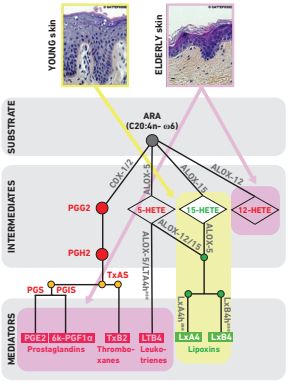


Figure 4. Proposed mechanistic model summarizing the age-associated alterations identified in skin explants.

Development of an *in vitro* model of lipid-driven skin aging

Multiple comparison analysis revealed age-specific lipid signatures (figure 5): LXA4, LXB4, and RvD2 were characteristic of young skin, whereas elderly skin was enriched in PGE2, 12-HETE, RvD1, and RvD3.

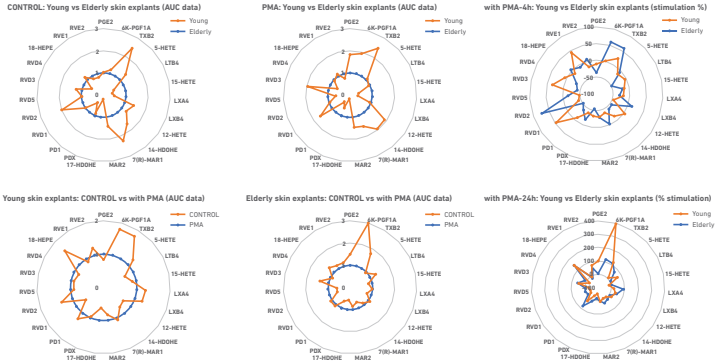
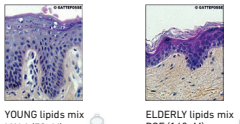


Figure 5. Bioactive lipid profiling in PMA-challenged skin explants from young and elderly donors: multiple-comparison analysis.

Based on these distinct profiles, we generated donor-representative lipid mixtures that reproduced the physiological concentrations and relative proportions observed in each group (figure 6).



YOUNG lipids mix
LXA4 (59nM)
LXB4 (59nM)
RvD2 (20nM)

ELDERLY lipids mix
PGE (142nM)
12-HETE (664nM)
RvD1 (20nM)
RvD3 (59nM)

Figure 6. Bioactive lipid mixtures representative of skin explants from young and elderly donors.

Lipid-driven aging impairs dermal cell homeostasis

In normal human dermal fibroblasts and monocyte-derived M0 macrophages, the young lipid mixture preserved or enhanced the resolution inflammation index, whereas the elderly mixture markedly impaired it, driven by a near-abolished 15-HETE production and increased levels of pro-inflammatory mediators (figure 7).

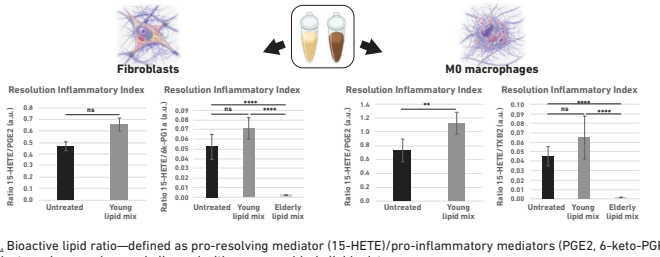


Figure 7. Bioactive lipid ratio—defined as pro-resolving mediator (15-HETE)/pro-inflammatory mediators (PGE2, 6-keto-PGF1α)—in fibroblasts and macrophages challenged with young or elderly lipid mixtures.

Fibroblasts exposed to the elderly mixture displayed strong MMP-3 induction at both mRNA (+146%, p<0.05*) and enzymatic activity levels (+51%, p<0.05*), while the young mixture significantly reduced MMP-3 expression (figure 8).

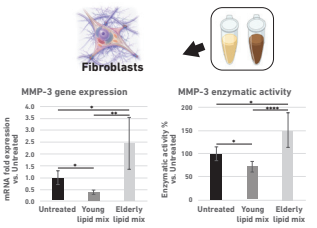


Figure 8. MMP-3 enzymatic activity and mRNA expression levels in fibroblasts challenged with young or elderly lipid mixtures.

CONCLUSION

Aging disrupts arachidonic acid pathway class-switching, shifting lipid mediator biosynthesis from pro-resolving lipoxins toward pro-inflammatory signaling, with a marked reduction of the lipoxin precursor 15-HETE in elderly skin.

Elderly lipid microenvironments impair the immunomodulatory functions of fibroblasts and macrophages, potentially altering their bidirectional crosstalk and promoting matrix degradation through MMP-3 induction.

Donor-specific lipid-reconstituted microenvironments provide a powerful experimental platform to model lipid-driven skin aging and dissect fibroblast–immune cell crosstalk underlying age-associated inflammatory dysfunction.