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Research Techniques Made Simple: Lipidomic Analysis in Skin Research

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Although lipids are crucial molecules for cell structure, metabolism, and signaling in most organs, they have additional specific functions in the skin. Lipids are required for the maintenance and regulation of the epidermal barrier, physical properties of the skin, and defense against microbes. Analysis of the lipidome—the totality of lipids—is of similar complexity to those of proteomics or other omics, with lipid structures ranging from simple, linear, to highly complex structures. In addition, the ordering and chemical modifications of lipids have consequences on their biological function, especially in the skin. Recent advances in analytic capability (usually with mass spectrometry), bioinformatic processing, and integration with other dermatological big data have allowed researchers to increasingly understand the roles of specific lipid species in skin biology. In this paper, we review the techniques used to analyze skin lipidomics and epilipidomics.

INTRODUCTION

The skin has a diverse lipid content, determined by its architecture, which comprises barrier and surface lipids and lipids of the epidermis and dermis. Dividing the lipids into such categories helps with understanding the biological function and also for a sampling of the analytes.

A lipid-based skin barrier is essential for terrestrial life. Barrier lipids, mainly ceramides with prevalent very long acyl chains, free fatty acids, and cholesterol in approximately 1:1:1 molar ratio, fill the extracellular spaces in the uppermost skin layer, the stratum corneum (Elias, 1983). This multilamellar, tightly packed lipid mixture forms an effective barrier to the environment, thus protecting the body against harmful external factors such as pathogens, allergens, chemical compounds, and UVR as well as restricting water loss. Not only the lipid composition but also their organization within the stratum corneum is essential for a proper skin barrier (barrier lipid organization probing is not covered in this Research Techniques Made Simple). Impaired composition, organization, and homeostasis of epidermal lipids are associated with a wide range of skin diseases (Bouwstra and Ponec, 2006).

The surface lipids derive from epidermal keratinocytes (KCs) and from sebum, a mixture of triglycerides, wax esters, squalene, and fatty acids produced by the sebaceous glands. Sebum moisturizes the skin and affects the skin immunological properties and microbiome in both homeostasis and disease (Lovászi et al., 2017). Because the skin surface and the stratum corneum are easily accessible with noninvasive or minimally invasive techniques, these lipids are the readily analyzed.

The inner, living skin layers lack barrier-specific lipids but contain higher amounts of phospholipids, the typical lipids present in cell membranes. Together with phospholipid metabolites, eicosanoids, endocannabinoids, and many other species, phospholipids mediate reactions to external insults such as UVR and pathogens. They generate intracellular, paracrine, or systemic signals and play significant roles in major inflammatory skin diseases (Kendall et al., 2015).

Because the dermal compartment is physically separated from the outside, dermal lipids are only targeted by select exogenous stimuli such as oxidizing long-wavelength UVR. Dermal lipids play active roles in inflammation, metabolism, aging, and wound healing (Gruber et al., 2019).

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Abbreviations: 2D, two-dimensional; AD, atopic dermatitis; DESI, desorption electrospray; HPTLC, high-performance thin-layer chromatography; KC, keratinocyte; LC, liquid chromatography; LC-MS, liquid chromatography coupled with mass spectrometry; LLE, liquid–liquid extraction; MALDI, matrix-assisted laser desorption/ionization; MS, mass spectrometry; MSI, mass spectrometry–based imaging; MS/MS, tandem mass spectrometry; SIMS, secondary ion mass spectrometry; TLC, thin-layer chromatography

SUMMARY POINTS

Advantages

- Skin lipidomic analysis is valuable in exploring skin homeostasis and disease and has ample translational potential.

- Skin lipidome is heterogeneous; localization and function are reflected in composition (surface, barrier, appendage).

- Analysis requires before isolation and extraction in organic solvents, and preanalytical separation is optional.

- The analytical methods differ in complexity, in their demands on infrastructure, and in the results they deliver.

- High-performance thin-layer chromatography (TLC) (HPTLC) provides a quick overview of the sample and basic quantification of lipid subclasses.

- Mass spectrometry (MS) detects a large number of lipid ions. Separation of the lipids can be either performed before the MS using liquid chromatography (LC) or executed within the ion source of shotgun analysis.

- MS-based imaging (MSI) performs spot-by-spot MS over the sample area.

- In TLC and HPTLC, there is low cost for equipment and a quick overview of lipid classes in a sample.

- In shotgun MS, there is high-throughput information on a wide range of lipids in the sample.

- In LC coupled with MS (LC-MS), there are the highest sensitivity, possibility to do targeted quantitative, and qualitative analyses.

- In MSI, there is a localization of numerous lipid species within a skin sample.

Limitations

- In TLC and HPTLC, there is little information beyond lipid class.

- In shotgun MS, there is low power on quantification and structural identification.

- In LC-MS, LC separation and data processing are time consuming.

- In MSI, there are expensive machinery, intensive human resource requirements, and low throughput.

SAMPLING

Collection of sebum, which is easy and noninvasive, can be done using adhesive or sebum-absorbing patches (Camera et al., 2016). Sebum lipids can also be retrieved from hairy body sites by cutting the hair and applying organic solvents

to the sample. Stratum corneum samples are usually obtained by horizontal sequential sectioning using adhesive tape (tape stripping [Masukawa et al., 2009]); however, a standardized methodology is still missing (reviewed in Keurentjes et al. [2021]). Sebum-derived surface lipids penetrate the stratum corneum to some extent, as was shown by lipidomics performed on serial tape strips (Sadowski et al., 2017). Cyanoacrylate (superglue) stripping and simple scraping with a scalpel are less mild, yet are straightforward methods of stratum corneum sampling. An important point to be considered is that adhesive materials used for sampling can contain lipids (e.g., fatty acids) that could contaminate the analytes. Alternatively, stratum corneum can be obtained by trypsinization from punch biopsies or ex vivo skin explants. For the full epidermal lipid spectrum, the epidermis can be separated from the dermis (i) in vivo using the suction blister technique (yielding interstitial fluid lipids that also can be analyzed) or (ii) ex vivo using heat separation or the enzyme dispase, which cleaves the basal membrane collagen IV. Free extractable (intercellular) stratum corneum lipids can be extracted by organic solvents, typically chloroform/methanol mixtures (Bligh and Dyer, 1959). The corneocyte lipid envelope (ultralong ceramides and fatty acids covalently bound to corneocyte envelope proteins) can be released from the remaining stratum corneum sheets by alkaline hydrolysis and extracted with an organic solvent. For dermal tissue biopsies, isolated and pelleted cells, or cultured cells, the typical extraction protocols are derived from the classical methods (Bligh and Dyer, 1959) and can then be followed by chromatographic separation or liquid–liquid extraction (LLE) methods to enrich specific lipid classes. For example, LLE with hexane/acidic methanol can efficiently enrich polar lipids (e.g., phospholipids) from cultured KCs, fibroblasts, melanocytes, and sebocytes (Gruber et al., 2012).

ANALYTICAL METHODS

Thin-layer chromatography and high-performance thin-layer chromatography

Thin-layer chromatography (TLC) is a basic and relatively inexpensive method for fast lipid profiling in a sample and must include lipid standards (Fuchs et al., 2011). This technique pioneered research on skin barrier lipid composition, metabolism, and pathological changes. In TLC, a mobile phase (a solvent mixture) passes over a thin layer of a stationary phase separating a lipid mixture into its components. In high-performance TLC (HPTLC), smaller and uniform stationary phase particles provide a higher level of separation than conventional TLC, thus increasing the resolution of the method. Separated lipids are typically visualized as dark spots by destructive derivatization using an acidic copper sulfate solution with heating (Figure 1). Specific derivatization (e.g., for glycolipids), functional group–specific reactions, and coupling to mass spectrometry (MS) also exist. For lipid identification and quantification, authentic standards must be run in parallel on the same TLC plate, providing an overview of several lipid classes. HPTLC delivers data that are comparable with results of liquid chromatography (LC) coupled with MS (LC-MS) on the level of the major lipid classes (and several but not all ceramide subclasses) (Masukawa et al., 2009; Ohno et al., 2017; t'Kindt et al., 2012), however without any further specification on their acyl chain length or stereochemistry.
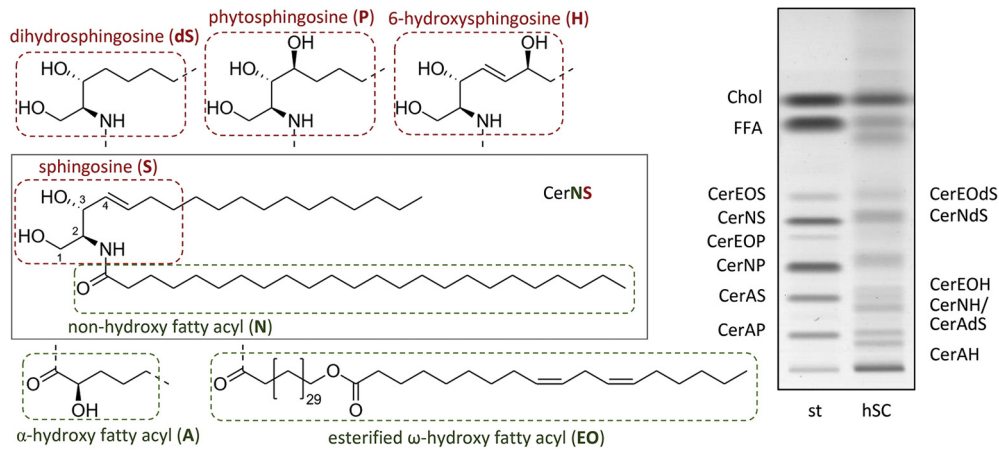
Figure 1. Exemplary HPTLC separation of skin barrier lipids. An HPTLC separation of lipid sts and extracted hSC lipids. sts lipids comprised Chol, FFA, and six Cer subclasses (their nomenclature based on structure is schematically shown [left]). The hSC lipids also contained 6-hydroxyCer (CerEOH, CerNH, CerAH), which are not commercially available, and dihydroCer (CerEOdS, CerNdS, CerAdS), which coelute with their saturated sphingosine-based counterparts (CerEOS, CerNS, CerAS, respectively). Cer, ceramide; CerAdS, α-Hydroxy FA [A] Dihydrosphingosine [DS] ceramide; CerAH, α-Hydroxy FA [A] 6-Hydroxy sphingosine [H] ceramide; CerAS, α-Hydroxy FA [A] Sphingosine [S] ceramide; CerEOdS, Esterified ω-hydroxy FA [EO] Dihydrosphingosine [DS] ceramide; CerEOH, Omega-hydroxy-fatty acid [EO] 6-hydroxy-sphingosine [H] ceramide; CerEOS, Esterified ω-hydroxy FA [EO] Sphingosine [S] ceramide; CerNdS, Non-hydroxy FA [N] Dihydrosphingosine [DS] ceramide; CerNH, Non-hydroxy FA [N] 6-Hydroxy sphingosine [H] ceramide; CerNS, Non-hydroxy FA [N] Sphingosine [S] ceramide; Chol, cholesterol; FFA, free fatty acid; HPTLC, high-performance thin-layer chromatography; hSC, human stratum corneum; st, standard.

Chromatography-coupled MS

MS lipidomics, which allows for detection of the mass-to-charge ratio for a large number of (ionizable) lipids, has become a tool of choice for addressing the complexity of the skin lipidome because sensitive high mass accuracy and resolution instrumentation (Fourier-transform ion cyclotron resonance, Orbitrap) and data processing software have been developed (Wang et al., 2016).

LC coupled on line to MS became one of the most popular tools in addressing the complexity of the skin lipidome.

Considering the large diversity of individual lipids species, each with its own functionality, LC-MS coupling significantly increases the power of the analytical platform and thus provides a sensitive and specific tool to identify and quantify skin lipids (e.g., barrier lipids [van Smeden et al., 2014]). For instance, application of reverse-phase chromatography allows for the separation of diverse subclasses of ceramide lipids on the basis of their hydrophobicity (t'Kindt et al., 2012) (Figure 2), whereas the free fatty acids must be often derivatized before their analysis (Norlén et al., 1998). High-mass accuracy and high-resolution MS, in turn, allow for a
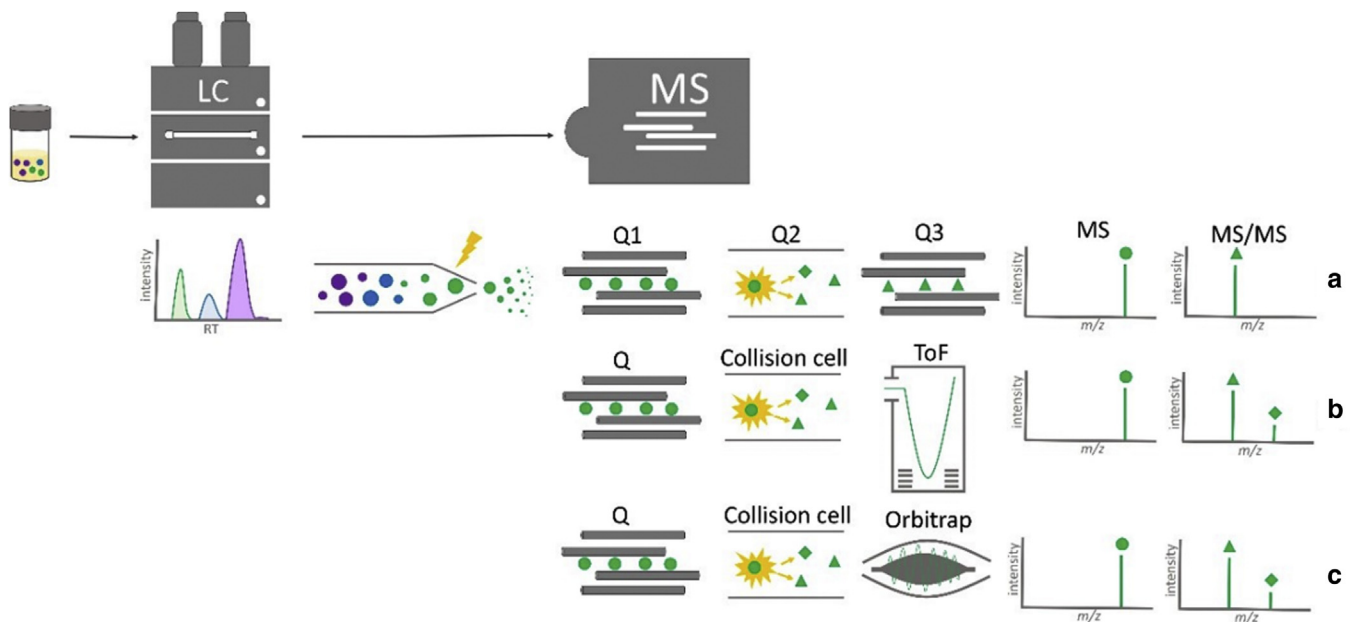

Figure 2. Schematic representation of the LC-MS/MS workflow. Workflow used for (**a**) targeted and (**b, c**) untargeted lipidomics. Extracted lipids are separated by LC (e.g., using reverse-phase or hydrophilic interaction chromatography) coupled on line to the mass spectrometer equipped with an electrospray ionization source. Ionized lipids can be analyzed using targeted methods such as (**a**) MRM or untargeted approaches (e.g., DDA) on high-resolution and high-mass accuracy hybrid instruments such as (**b**) QTOF and (c) quadrupole Orbitrap. DDA, data-dependent acquisition; LC, liquid chromatography; MRM, multiple reaction monitoring; MS, mass spectrometry; MS/MS, tandem mass spectrometry; QTOF, quadrupole-time of flight; ToF, time of flight.
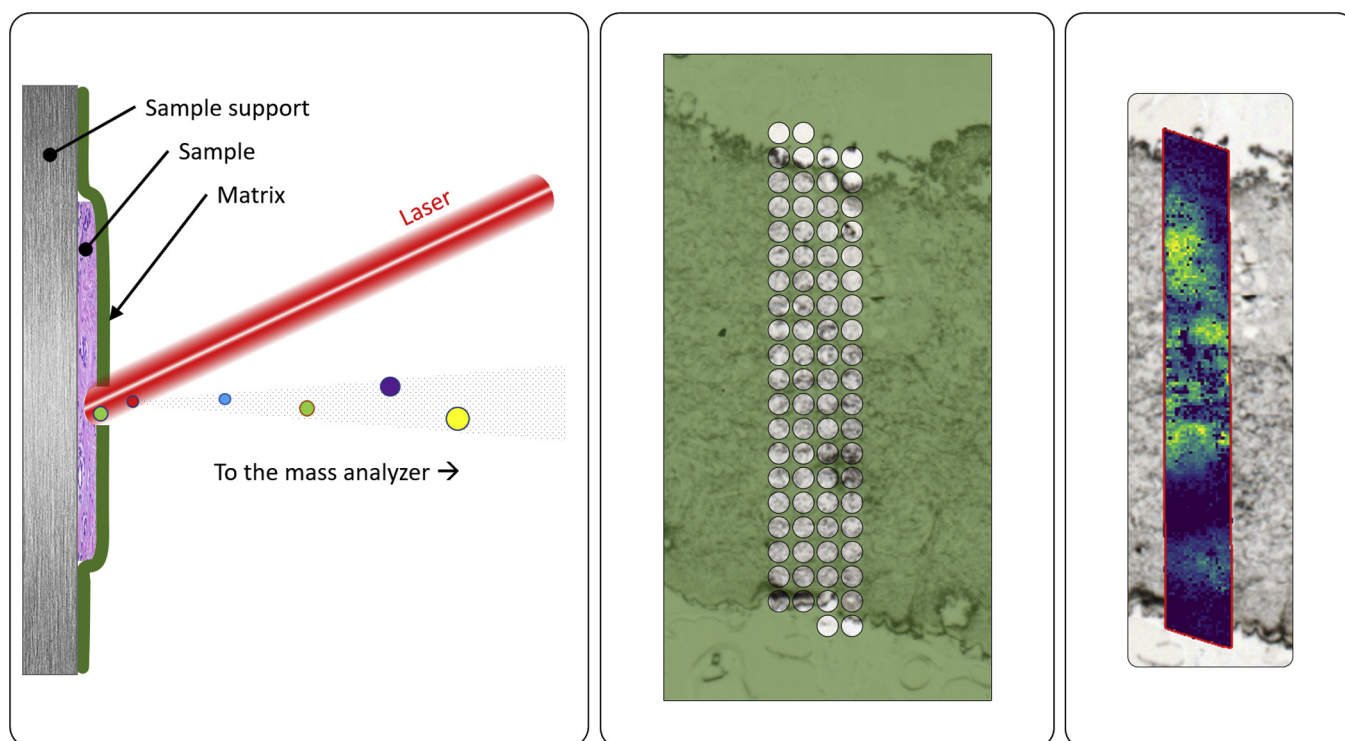
Figure 3. Schematic representation of a MALDI MSI experiment on a skin biopsy. Left panel: The sample is mounted on sample support, which is then covered with the matrix, by sublimation or by spraying. The energy of the laser beam is absorbed by the matrix and ionizes the analyte, which is then accelerated into the mass analyzer. Middle panel: Schematic representation of the laser beam–targeted sampling sites (lateral resolution with MALDI MSI down to 10 μm). Right: a typical result of a MALDI–MSI analysis of a skin section; color scheme indicates the TIC normalized relative to the intensity distribution for one ion from 0% (black) to 100% (yellow). MALDI, matrix-assisted laser desorption/ionization; MSI, mass spectrometry–based imaging; TIC, total ion count.

determination of the exact m/z (mass-to-charge ratio) of ionized lipid species and when used in tandem MS (MS/MS) mode, can provide fragment spectra (MS/MS) suitable for accurate identification of lipid molecular species. For all the MS methods discussed, the resolving power and accuracy of mass measurement are critical to unambiguously distinguish lipids of very similar m/z. The latest generation instruments fulfill this demand, having high mass resolution (m/Δm>1 Mio) and accuracy ((m$_{meas.}$−m$_{theor.}$)*10^6/m$_{theor.}$<1 ppm).

LC-MS/MS data can be acquired in untargeted (to describe whole-lipidome complexity and support lipids relative or accurate quantification) or targeted (for absolute quantification) modes. Targeted LC-MS/MS analysis is suitable for high-throughput quantification of selected lipids in large sample cohorts with minimal data processing, but previous knowledge of the lipids of interest is required, including their defined structures, elemental composition, m/z, and fragmentation patterns (Figure 2a). Untargeted lipidomics provides a more holistic view of the lipidome in question but usually requires intensive data processing starting with the identification of individual lipid species from the fragment spectra obtained in MS/MS experiments (Figure 2b and c).

The high resolving power of LC-MS/MS is ideal for addressing the new field of epilipidomics and the investigation of oxidative or other chemical modifications of lipids that change their chemical and biological properties. These modifications add another layer of complexity to the analysis, but several studies have shown the importance of the epilipidome in a plethora of physiological and pathophysiological processes, also

in the skin (reviewed in Kendall et al. [2018] and Gruber et al. [2020]).

Shotgun lipidomics
Shotgun lipidomics omits time-consuming chromatographic separation, instead of using separation techniques within the ion source that separate predetermined groups of lipid classes on the basis of their electrical characteristics (Han and Gross, 2005). Shotgun lipidomics provides high-throughput data of lipidomic signatures, which can serve as biomarkers of skin homeostasis or disease (Franco et al., 2018). Stratum corneum and epidermal and cellular lipid compositions have been successfully identified using shotgun lipidomics (Sadowski et al., 2017).

Mass spectrometric imaging
MS-based imaging (MSI) is a powerful tool to monitor multiple analytes within a tissue (Marchetti-Deschmann et al., 2021) in a single experiment. MSI allows for label-free detection, identification, and to some extent, quantification of skin lipids. Spot-focused MS measurements in a two-dimensional (2D) array on thin tissue sections (5–10μm) use ionization techniques such as matrix-assisted laser desorption/ionization (MALDI), secondary ion mass spectrometry (SIMS), or desorption electrospray (DESI). Although the prospect of having a molecular/histological analysis of skin and its lipids is very enticing, there are technical challenges. The spatial resolution for SIMS is 100–500 nm, limiting the analysis to rather small areas. MALDI and DESI are effective at 10–20 μm and 50–100 μm, respectively, limiting subcellular information. Shotgun analysis ionization efficiencies for the different

lipid classes and ion suppression effects hamper sensitivity. The homogeneous application of a proper matrix is important for MALDI. Nevertheless, MSI has been used to investigate skin lipid distribution, wound healing, and skin aging and to validate skin organotypes (reviewed in Gruber et al. [2020] and Narzt et al. [2021]) (Figure 3).

MS DATA PROCESSING AND INTEGRATION
Untargeted lipidomic experiments result in several thousand MS/MS spectra. Therefore, interpretation is laborious and does not support the high-throughput rates expected from modern omics technologies. Thus, bioinformatics tools capable of providing software-assisted lipid identification are usually implemented for the analysis of lipidomics data. Over the last years, numerous tools were developed by MS vendors and academic groups to support high-throughput lipid identification, including LipidHunter (Ni et al., 2017), Lipostar (Goracci et al., 2015), MS DIAL (Tsugawa et al., 2020), and others. Lipid identification software capabilities are as follows: (i) software capable of matching experimental MS/MS data to pre-existing spectra libraries acquired from lipid standards or generated in silico (e.g., MS DIAL), (ii) software using curated fragmentation rules established for different lipid classes for bottom–up lipid identification on the basis of the fragment ion signals in acquired MS/MS (e.g., LipidHunter), and (iii) software that combines both of these capabilities (e.g., Lipostar). Lists of accurately identified lipid molecular species can be used for quantitative analysis and systems biology integration of the lipidome profiles obtained.

PATHWAY AND NETWORK ANALYSIS
Lipidomics data can be considered to be a subset of metabolomics, but the identification of individual lipids is more complicated than that of more general metabolites. Identification of individual lipids is crucial for pathway and network analyses, not only to connect the lipidomics data to pathway models and molecular networks but also to integrate with other omics data. Identifier mapping tools such as BridgeDb enable automated mapping between identifiers from different databases (van Iersel et al., 2010), allowing simple manual and automated analysis and visualization of lipidomics data on biological pathway models from Wiki-Pathways (Martens et al., 2021) (lipid portal: http://lipids.wikipathways.org/) using PathVisio (https://pathvisio.github.io), a freely available pathway editor, visualization, and analysis software. Finally, automated data analysis in R and other programming languages can also be easily extended with pathway and network approaches in Cytoscape (http://cytoscape.org), a widely adopted network analysis and visualization platform.

MODEL SYSTEMS
Some experiments cannot be performed in vivo in humans owing to either ethical reasons or tissue complexity. Thus, often, a suitable model must be used. Whereas mouse remains a widely used model organism with valuable established methods for genetic modification and subsequent analysis of skin and epidermal lipids, human tissue–derived models are required for validation and species-specific investigation. Isolated and 2D-cultured (human) skin cells (e.g., healthy or from lesional areas) provide material for high-throughput scanning/analysis shotgun lipidomics and untargeted LC-MS and bring insight into disease-specific dysregulation of skin lipid metabolism (Han and Gross, 2005). Nevertheless, isolated cells lack the crosstalk with other skin compartments, which is partially eliminated in three-dimensional skin constructs. Organotypic models contain living cells, which after air exposure develop a stratified epidermal structure and are suitable to address how external stimuli or genetic manipulation affect lipids in the epidermal or dermal compartments (van Drongelen et al., 2013; Vávrová et al., 2014). Insights gained in these models are limited by potential confounding effects through components of the growth medium or culture conditions (Thakoersing et al., 2015) and by observed differences in lipid composition and barrier functionality between skin construct and native tissue (Thakoersing et al., 2013; van Smeden et al., 2014) (reviewed in [Bouwstra et al., 2021]).

The effects of specific lipid alterations on skin barrier properties and function can be studied using acellular models of the stratum corneum lipid matrix. Although the simplest of such models are useful for probing specific lipid behavior details with nuclear magnetic resonance, infrared spectroscopy, X-ray, or neutron diffraction, more complex models reasonably mimic stratum corneum lipid lamellar and lateral architecture, chain order, and permeability (de Jager et al., 2006).

SKIN LIPID ANALYSIS IN BASIC, CLINICAL, AND TRANSLATIONAL DERMATOLOGICAL RESEARCH
Lipidomics identified the roles of bioactive lipid mediators in the skin in inflammation (Kiezel-Tsugunova et al., 2018), photobiology and stress (Gruber et al., 2019), and aging and senescence (Narzt et al., 2021); their distribution in the skin (Kendall et al., 2015); and how diet affects their composition and immunomodulatory action (Kendall et al., 2019).

The example of atopic dermatitis (AD) probably best shows how lipidomic analysis supports progress in basic and applied dermatological research. Thanks to the lipid analysis (originally TLC and later LC-MS), lipid alterations were correlated with impairment of barrier functions in various skin diseases, including AD (Bouwstra and Ponec, 2006). Analysis of skin samples from patients with AD revealed changes in the amount and composition of skin barrier lipids (Ishikawa et al., 2010). In addition, decreased expression of epidermal differentiation–related molecules, such as FLG, loricrin, and involucrin, had been shown in patients with AD. Because loss-of-function mutation in FLG gene is one of the strongest genetic risks for AD development, an effect of FLG mutations on skin barrier lipids was studied using FLG-knockdown skin constructs. Several studies were unable to show a direct correlation of FLG mutations or deletion with impaired barrier lipid content in the reconstructed epidermis (van Drongelen et al., 2013; Vávrová et al., 2014). However, lipidomic analysis on reconstructed epidermal equivalents derived from the KCs of patients with AD indicated a link between the FLG loss-of-function mutations and altered eicosanoid metabolism (Blunder et al., 2017), and changes in the eicosanoid

MULTIPLE CHOICE QUESTIONS

1. The skin barrier lipids mainly consist of the following

   A. Triglycerides, wax esters, squalene, and fatty acids

   B. Phospholipids

   C. Ceramides, free fatty acids, and cholesterol

   D. Glucosylceramides, sphingomyelin, phospholipids, and cholesteryl sulfate

2. What is the advantage of shotgun lipidomics?

   A. The data processing does not need elaborate software.

   B. High throughput because no separation of the sample before analysis is needed.

   C. The only technology that allows for the acquisition of targeted data and absolute quantification.

   D. Nobody dares to touch your mass spectrometer.

3. What information is crucial for pathway and network analysis on lipidomics data?

   A. The cellular location of lipid classes.

   B. Programming experience.

   C. Statistical preprocessing of the acquired data.

   D. Identifiers for individual lipids.

4. What is epilipidomics?

   A. The investigation of skin surface lipids.

   B. Application of an ionizable matrix onto a sample before laser ionization and mass detection.

   C. The research on modifications of the lipidome.

   D. Lipidomics on purified epidermis (after suction blister separation, heat separation, or enzymatic digestion)

5. Thin-layer chromatography…

   A. is environmentally friendly because it does not need any solvents.

   B. can detect surface lipids but not ceramides.

   C. can be used to quantify lipids when authentic standards are available.

   D. detects lipid chain fluidity and packing.

analysis in pediatric patients with AD (Joo et al., 2015). Lipidomics of patient or model system material are therefore very useful to investigate hypotheses regarding the effect of specific genes on the pathology-associated lipidome changes (e.g., structural lipids or eicosanoid inflammatory mediators). Therefore, lipidomics enhances our understanding of barrier, immunological, dietary, developmental, and systemic versus local aspects of AD and its pathogenesis by providing novel immunomodulatory lipid mediators and represents a tool for evaluation of various therapeutic approaches, for example, effect of dietary interventions on the proinflammatory status in patients with AD.

Lipidomics gain importance in (skincare) industry-driven research and serve as valuable targets to assess the efficacy of both cosmetic formulation and skincare active ingredients (Letsiou et al., 2020). In cosmetic or personal care formulations oils, fats, waxes, and phospholipids are employed as excipients, and lipid antioxidants such as carotenoids, retinoids, and tocopherols are used for their antioxidant properties and biological effects, and these lipid ingredients require lipidomic analyses for efficacy and quality control.

CONCLUSION

In conclusion, lipids play crucial roles in maintaining the skin barrier, supporting skin structure, providing defense against external attack, maintaining the microbiome, as well as protecting against disease-causing pathogens and regulating inflammation. A systematic integration of the detailed molecular characterization of lipids and their signaling, biochemical, and biophysical properties, with their localization at high resolution within the skin, promises to provide researchers with a systems view of the roles of lipids in this lipid-rich organ.

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composition have been reported, both in the serum and skin of patients with AD (Töröcsik et al., 2019). Furthermore, the discrimination between healthy and atopic phenotype in mouse skin based on relative quantification of selected sphingosine ceramides by shotgun methodology has been reported (Franco et al., 2018). The same ceramide classes were correlated with disease severity using targeted LC-MS

RESEARCH TECHNIQUES MADE SIMPLE

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SUPPLEMENTARY MATERIAL
Supplementary material is linked to this paper. Teaching slides are available as supplementary material.

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RESEARCH TECHNIQUES MADE SIMPLE

DETAILED ANSWERS

1. The skin barrier lipids mainly consist of the following

CORRECT ANSWER: C. Ceramides, free fatty acids, and cholesterol.

Ceramides, free fatty acids, and cholesterol in an approximately 1:1:1 molar ratio fill the extracellular spaces in the skin uppermost layer, the stratum corneum. These lipids, tightly packed into lamellae, restrict water loss and prevent penetration of potentially harmful compounds from the environment.

2. What is the advantage of shotgun lipidomics?

CORRECT ANSWER: B. High throughput because no separation of the sample before analysis is needed.

Identification can be achieved by performing class separation at the ion source; the chromatography is omitted, resulting in drastically shortened run times. Chromatographic separation before the mass spectrometry (MS) is however indicated when quantification and structural information are required.

3. What information is crucial for pathway and network analysis on lipidomics data?

CORRECT ANSWER: D. Identifiers for individual lipids.

Clear identifiers are needed to integrate lipid (and other omics) data into pathways and networks.

4. What is epilipidomics?

CORRECT ANSWER: C. The research on modifications of the lipidome.

Epilipidomics describes the new field of research investigating oxidative or other chemical modifications of lipids that change their chemical and biological properties, which has been made possible by high-resolution MS methods.

5. Thin-layer chromatography…

CORRECT ANSWER: C. can be used to quantify lipids when authentic standards are available.

Standards can be used to construct calibrations curves and quantify the lipids separated by thin-layer chromatography.