Lipidomics needs more standardization

Modern mass spectrometric technologies provide quantitative readouts for a wide variety of lipid specimens. However, many studies do not report absolute lipid concentrations and differ vastly in methodologies, workflows and data presentation. Therefore, we encourage researchers to engage with the Lipidomics Standards Initiative to develop common standards for minimum acceptable data quality and reporting for lipidomics data, to take lipidomics research to the next level.

Lipidomics Standards Initiative Consortium

Lipidomics has evolved rapidly over the past decade because it offers new opportunities for studying the roles of lipids in cellular biology as well as in health and disease[1]. The lipidomes of eukaryotic cells comprise hundreds of individual lipid species that structurally and chemically regulate cell membrane dynamics, store energy and/or serve as precursors of bioactive metabolites[2]. Membranes of cells and organelles have unique lipid compositions that are intimately linked to their biological functions. The biophysical properties of membranes are also affected by seemingly minor structural differences among individual lipid species, such as the number, position and geometry of double bonds in acyl chains. These characteristics drive membrane budding and fission events and may regulate protein function[3]. Lipid species in membranes act not as single molecules but as a collective, and must be analysed quantitatively and comprehensively to understand their biological function. Examples of bioactive lipid species include typical membrane lipids, such as ceramide d18:1/16:0 (a selective natural ligand of p53; ref. [4]) or fatty-acid-derived pro-inflammatory mediators (for example, prostaglandins and leukotrienes) and anti-inflammatory mediators (for example, resolvins, protectins and maresins)[5]. The power of lipidomics is further demonstrated by the identification of ceramide species as risk markers for cardiovascular disease in independent large-scale lipidomics studies[6,7]. These and other intriguing results have spurred interest in adopting lipidomics capabilities across research communities.

A major challenge in the field of lipidomics, however, is the large disparity in methodologies and technologies, which has resulted in discrepancies in published data and broader issues of irreproducibility[8]. Common problems include improper annotation of lipid species (despite the publication of an accurate shorthand annotation for lipid species in 2013; ref. [9]), misidentifications and over-reporting, which are probably caused by incorrect mapping of mass-spectral features to potential lipid molecules, owing to software errors combined with a lack of manual data inspection or curation. Similarly, the data are commonly reported in arbitrary units (usually ion counts of peak intensity or area) even though quantification of molecule numbers (such as moles) is necessary for calculation of the fractions for lipid classes and species, and is the only adequate solution for detailed interpretation and comparison of datasets. Detailed structural analysis of lipids, such as the identification of double-bond positions in acyl chains, is needed for functional decoding of individual lipids to advance lipid biology.

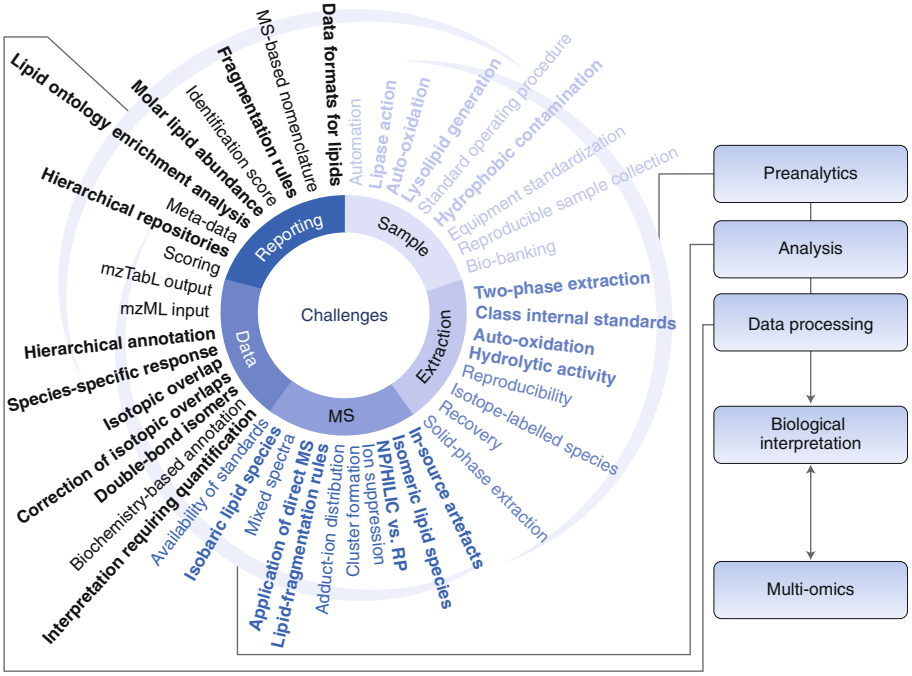

Fig. 1 | Analytical challenges in lipidomics workflows. Terms in bold are particularly important in lipidomics workflows. MS, mass spectrometry; NP/HILIC, normal phase/hydrophilic-interaction liquid chromatography; RP, reversed-phase.

Why we need standards for lipidomics

An inter-laboratory comparison involving the quantification of lipids in human plasma has demonstrated that the diversity of lipid analysis strategies is reflected in the variations in concentrations of the measured lipid species[10]. We believe that a community-wide, open discussion of the methods used and of how lipidomics data are presented is needed to achieve accurate quantification and reproducibility of results. This effort should identify issues, as mentioned above, in lipidomics workflows and develop guidelines for the entire lipidomics process, from preanalytics, lipid extractions, mass spectrometric analysis, data analysis and reporting. One such initiative specifically for the

lipidomics analysis of human plasma is already ongoing[11].

Such guidelines should be adapted, as appropriate, from existing 'omics' guidelines[12,13]. However, lipidomics differs in certain aspects from other omics strategies and thus requires its own set of standards[14] (Fig. 1). One advantage of lipidomics, compared with other omics fields, is that the fragmentation pathways for most existing lipid classes are known, thereby allowing rules to be defined for the identification of lipid species rather than relying on spectral similarities between lipids. Thus, lipid identification can be improved by annotating lipids correctly in accordance with the obtained mass spectrometry data[9] and through the use of internal standards, which allow for accurate quantification[14].

Lipidomics analyses are particularly challenging because of the richness in isomeric species, mainly resulting from variations in acyl-chain length and the positions of double bonds. For example, lipids that differ merely in the number of double bonds generate substantial isotopic overlap particularly resulting from ^{13}C atoms. Thus, the $M + 2$ isotopic peak for a typical phospholipid is higher than 10% related to its monoisotopic peak and overlaps with a species containing one fewer double bond. Lipids are also prone to artefacts as a result of in-source fragmentation, including during sampling by autoxidation and action of lipases[8]. Although semiquantitative approaches may be applicable for biomarker discovery or may provide valid data on relative changes in lipid species, we believe that lipidomics methods must allow for quantitative analysis to study the interplay among lipids in biological membranes. However, the quantification of a large number of lipid species requires tailored approaches.

The Lipidomics Standards Initiative

The Lipidomics Standards Initiative (LSI; https://lipidomics-standards-initiative.org/) was launched in spring 2018 to address these challenges. Since then, the LSI has participated in several workshops and conferences to propose the introduction of guidelines and standards for lipidomics, which aim to improve the overall understanding of analytical chemistry (mass spectrometric analysis) and lipid biology, and should be particularly useful to researchers new to the lipidomics field. We believe that it is time to increase awareness of the LSI, not only within the lipidomics community but also among metabolomics researchers working in related disciplines who produce lipid datasets.

Importantly, our commitment is to align the LSI with existing initiatives to develop guidelines for lipidomics. We have established a collaboration with LIPID MAPS (https://www.lipidmaps.org/), are currently discussing an adaptation of mzTab[15] to report lipidomics data, and have started an active dialog with other initiatives and communities. For instance, the LSI promotes development of lipo-centric hierarchical databases, such as SwissLipids (http://www.swisslipids.org/) and LipidHome (https://www.ebi.ac.uk/metabolights/lipidhome/), as well as search tools, such as 'bulk' structure searches of LIPID MAPS (https://www.lipidmaps.org/resources/tools/bulk_structure_searches_overview.php).

The LSI homepage contains the first drafts of guidelines covering all steps of the most common lipidomics workflows (https://lipidomics-standards-initiative.org/guidelines) in an effort to stimulate discussion and to promote their development. The LSI is outward facing in that anyone can directly communicate with the LSI community through discussion boards on the homepage, an interaction that we highly encourage. The guidelines on the LSI website include the various analytical steps in a lipidomics workflow, providing guidance on how to (i) collect and store samples; (ii) extract lipids; (iii) execute the mass spectrometry analysis; (iv) perform data processing, including lipid identification, deconvolution, annotation, quantification and evaluation of quality control; and (v) report the data. The guidelines also cover the validation of analytical methods and the use of quality controls. Failure to follow a set of sufficient rules or guidelines increases the likelihood of errors during all stages of the lipidomics workflow, thus potentially leading to data irreproducibility and incorrect reporting or interpretation. Therefore, LSI aims to provide a checklist to guide users in how to achieve a minimum acceptable level of data quality and to inform editors and reviewers who evaluate manuscripts containing lipidomics data.

Conclusions

Guidelines regarding standards for lipidomics are needed to unlock the full potential of lipidomics. Such guidelines will be key in enabling lipidomics to meet regulatory requirements for use in clinical research and diagnostics. For translation to clinical diagnostics, lipidomics methods must be validated to comply with US Food and Drug Administration and European Medicines Agency requirements. In basic research, lipidomics standards should enhance the comparability of data and, together with resources of lipid-species profiles for specific biological materials including human and murine body fluids and tissues, these standardization efforts should enhance understanding of the functional roles of specific lipid species.

Here, we report the first steps toward the urgently needed standardization of lipidomics. With this first draft of guidelines, we make a strong appeal to the community to engage with the LSI in facilitating implementation and continuous development of standards. We encourage researchers to use our discussion board or to connect directly with individual LSI members. With this initiative, we also aim to channel the development of lipidomics and make it more effective, and we welcome interactions and sharing of data standards with other disciplines and initiatives. Starting this standardization process now is important because the number of lipidomics users, applications and methods is rapidly growing, and any delay is likely to hamper the broad adoption of standards. ❐

Lipidomics Standards Initiative Consortium

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Author contributions

G.L. and K.E. prepared the manuscript and developed the online tool. J.A.B., W.J.G., R.A., T.W.M., Masanori Arita, Makoto Arita, M.R.W., C.S.E., H.K. and M.H. discussed and contributed to the manuscript and content of the online tool. All authors annotated data and approved the final manuscript.

Competing interests

K.E. is the owner of Lipidomics Consulting Ltd. The other authors declare no competing financial interests.