Lipidomics need more standarization

Lipidomics Standards Initiative Consortium*

Summary

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 appeal to 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.

Current state of lipidomics

Lipidomics has evolved rapidly over the past decade as it offers new opportunities for studying the role of lipids in cellular biology as well as in health and disease [1]. Lipidomes of eukaryotic cells comprise hundreds of individual lipid species that structurally and chemically regulate cell membranes dynamics, store energy, and/or serve as precursors of bioactive metabolites [2]. Membranes of cells and organelles have unique lipid compositions which are intimately linked to their biological functions. The biophysical properties of membranes are also affected by seemingly minor structural differences between individual lipid species, such as the number, position and geometry of double bonds in acyl chains. These characteristics drive membrane budding/fission events and may regulate protein function [3]. Lipid species in membranes act not as single molecules but as a collective which needs to be analyzed quantitatively and comprehensively to understand their biological function. Examples of bioactive lipid species include typical membrane lipids, such as ceramide (Cer) d18:1/16:0 as a selective natural ligand of p53 [4], or fatty acid-derived pro-inflammatory (i.e. prostaglandins, leukotrienes) and anti-inflammatory (i.e. resolvins, protectins, maresins) mediators [5]. The power of lipidomics is further demonstrated by the identification of ceramide species as risk markers for cardiovascular disease from 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, resulting 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 [9]), misidentifications and overreporting, likely caused by incorrect mapping of mass spectral features to potential lipid molecules due to software errors combined with the a lack of manual data inspection or curation. Similarly, the reporting of data in arbitrary units (usually ion counts of peak intensity or area) is commonly used even though only quantification of molecule numbers (*i.e.*, mol) allows calculating the fractions for lipid classes and species and being the only adequate solution for detailed interpretation and comparison of data sets. Detailed structural analysis of

lipids, such as the identification of double bond positions in acyl chains, are needed for a functional decoding of individual lipids to advance lipid biology.

Why we need standards for lipidomics

An inter-laboratory comparison involving the quantification of lipids in human plasma demonstrated that the diversity of lipid analysis strategies is reflected in the variation of concentrations of the measured lipid species [10]. We believe that a community-wide, open discussion of the methods used and how lipidomics data are presented is needed to achieve accurate quantification and reproducibility of results. This effort should identify issues, as mentioned above, in lipidomic workflows and develop guidelines for the entire lipidomic process, from preanalytics, lipid extractions, mass spectrometric analysis, data analysis and reporting. One such initiative is already ongoing specifically for the lipidomic analysis of human plasma [11].

Such guidelines should be adapted, where appropriate, from existing "omics" guidelines [12,13]. However, lipidomics differs in certain aspects from other omics strategies so that it requires its own set of standards [14] (**Figure 1**). One advantage, compared to other "omics" fields, is that the fragmentation pathways for most of the existing lipid classes are known. This allows us to define rules 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].

Lipidomic analyses are particularly challenging due to the richness in isomeric species, mainly resulting from variations in acyl chain length and the position 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 above 10% related to its monoisotopic peak and overlaps with a species containing one double bond less. Lipids are also prone to artifacts as a result of in-source fragmentation, including during sampling by autoxidation and action of lipases [8]. Although semi-quantitative approaches may be applicable for biomarker discovery or provide valid data on relative changes of lipid species, we think lipidomic methods need to allow for quantitative analysis in order to study the interplay of lipids in biological membranes. However, the quantification of a large number of lipid species requires tailored approaches.

Figure 1. Analytical challenges in lipidomics workflows. Bold terms are of particular importance for lipidomic workflows.

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 we hope will improve the overall understanding of analytical chemistry (mass spectrometric analysis) and lipid biology, which should be particularly useful to researchers that are new to the lipidomics field. We believe it is time to increase the awareness of the LSI, not only within the lipidomics community, but also among metabolomics researchers working in related disciplines who produce lipid data sets.

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 lipidomic data, and have started an active dialogue with other initiatives and communities. For instance, the LSI promotes development of lipo-centric hierarchical databases like SwissLipids (http://www.swisslipids.org/) and LipidHome (https://www.ebi.ac.uk/metabolights/lipidhome/) as well as search tools like "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 which cover all steps of the most common lipidomic 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 we highly encourage. The guidelines found on the LSI website include the various analytical steps in a lipidomics workflow, guiding how to (i) collect and store samples, (ii) extract lipids, (iii) execute the MS analysis, (iv) perform data processing, including lipid identification, deconvolution, annotation, quantification, and evaluation of quality control, and (v) how to report the data. The guidelines also cover the validation of analytical methods and the use of quality controls. Lack of, or failure, in following a set of rules or guidelines, increases the likelihood of errors occurring during all stages of the lipidomics workflow, 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, as well as reviewers, who evaluate manuscripts containing lipidomic data.

Conclusions

Guidelines about standards for lipidomics are required to unlock the full potential of lipidomics. Such guidelines will be key for lipidomics to meet regulatory requirements in order for lipidomics to be used in clinical research and diagnostics. In order to transit to clinical diagnostics, validation of lipidomics methods need to comply to FDA and EMA requirements. In basic research, lipidomics standards will enhance the comparability of data and, combined with resources of lipid species profiles for specific biological materials including human and murine body fluids and tissues, these standardization efforts will enhance our understanding of the functional roles of specific lipid species.

Taken together, we report the first steps towards urgently needed lipidomics standardization. Posting a first draft of guidelines represents a strong request to the community to engage with the LSI in order to facilitate implementation and continuous development of standards. We encourage researchers to use our discussion board or to connect directly to one of the LSI members. Embarking on lipidomics standardization now, represents the unique chance to introduce guidelines in an emerging community. Missing this opportunity will waste resources and hamper the broad adoption of standards due to a rapidly growing number of lipidomics users, applications and methods.

Acknowledgements

Work in Swansea was supported by the UK Biotechnology and Biological Sciences Research Council (BBSRC, grant number BB/N015932/1).
Work in Dortmund was supported by the Ministerium für Kultur und Wissenschaft des Landes Nordrhein-Westfalen, the Regierende Bürgermeister von Berlin - inkl. Wissenschaft und Forschung, and the Bundesministerium für Bildung und Forschung (de.NBI program code 031L0108A).
Work in Regensburg was supported by the European Union's FP7 programme MyNewGut (grant agreement number 613979) and Deutsche Forschungsgemeinschaft (DFG, LI 923/4-1).
Work in Pardubice was supported by the Czech Science Foundation (18-12204S).
Work in Japan was supported by MEXT KAKENHI (15H05897) and NBDC Integration Project.
Work in Graz was supported by the Austrian Federal Ministry of Education, Science and Research grant number BMWFW-10.420/0005-WF/V/3c/2017.
Work in Australia was supported by the Australia Research Council (DP150101715 and DP190101486)

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., M.A., 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 of the final manuscript.

Competing interests

Kim Ekroos is the owner of Lipidomics Consulting Ltd. The authors declare no competing financial interests.

References

1 Yang, K. & Han, X. Lipidomics: Techniques, Applications, and Outcomes Related to Biomedical Sciences. *Trends Biochem Sci* **41**, 954-969, doi:10.1016/j.tibs.2016.08.010 (2016).

2 van Meer, G., Voelker, D. R. & Feigenson, G. W. Membrane lipids: where they are and how they behave. *Nat.Rev.Mol.Cell Biol.* **9**, 112-124 (2008).

3 Ernst, R., Ejsing, C. S. & Antonny, B. Homeoviscous Adaptation and the Regulation of Membrane Lipids. *J Mol Biol* **428**, 4776-4791, doi:10.1016/j.jmb.2016.08.013 (2016).

4 Fekry, B. *et al.* C16-ceramide is a natural regulatory ligand of p53 in cellular stress response. *Nat Commun* **9**, 4149, doi:10.1038/s41467-018-06650-y (2018).

5 Serhan, C. N. & Levy, B. D. Resolvins in inflammation: emergence of the pro-resolving superfamily of mediators. *J Clin Invest* **128**, 2657-2669, doi:10.1172/JCI97943 (2018).

6 Havulinna, A. S. *et al.* Circulating Ceramides Predict Cardiovascular Outcomes in the Population-Based FINRISK 2002 Cohort. *Arterioscler Thromb Vasc Biol* **36**, 2424-2430, doi:10.1161/ATVBAHA.116.307497 (2016).

7 Sigruener, A. *et al.* Glycerophospholipid and sphingolipid species and mortality: the Ludwigshafen Risk and Cardiovascular Health (LURIC) study. *PLoS One* **9**, e85724, doi:10.1371/journal.pone.0085724 (2014).

8 Liebisch, G., Ekroos, K., Hermansson, M. & Ejsing, C. S. Reporting of lipidomics data should be standardized. *Biochim Biophys Acta Mol Cell Biol Lipids* **1862**, 747-751, doi:10.1016/j.bbalip.2017.02.013 (2017).

9 Liebisch, G. *et al.* Shorthand notation for lipid structures derived from mass spectrometry. *J Lipid Res* **54**, 1523-1530, doi:10.1194/jlr.M033506 (2013).

10 Bowden, J. A. *et al.* Harmonizing lipidomics: NIST interlaboratory comparison exercise for lipidomics using SRM 1950-Metabolites in Frozen Human Plasma. *J Lipid Res* **58**, 2275-2288, doi:10.1194/jlr.M079012 (2017).

11 Burla, B. *et al.* MS-based lipidomics of human blood plasma: a community-initiated position paper to develop accepted guidelines. *J Lipid Res* **59**, 2001-2017, doi:10.1194/jlr.S087163 (2018).

12 Orchard, S., Hermjakob, H. & Apweiler, R. The proteomics standards initiative. *Proteomics* **3**, 1374-1376, doi:10.1002/pmic.200300496 (2003).

13 Sumner, L. W. *et al.* Proposed minimum reporting standards for chemical analysis Chemical Analysis Working Group (CAWG) Metabolomics Standards Initiative (MSI). *Metabolomics.* **3**, 211-221 (2007).

14 Holcapek, M., Liebisch, G. & Ekroos, K. Lipidomic Analysis. *Anal Chem* **90**, 4249-4257, doi:10.1021/acs.analchem.7b05395 (2018).

15 Hoffmann, N. *et al.* mzTab-M: A Data Standard for Sharing Quantitative Results in Mass Spectrometry Metabolomics. *Anal Chem* **91**, 3302-3310, doi:10.1021/acs.analchem.8b04310 (2019).

* Lipidomics Standards Initiative Consortium

Gerhard Liebisch[1], Robert Ahrends[2], Makoto Arita[3], Masanori Arita[4], John A. Bowden[5], Christer S. Ejsing[6], William J. Griffiths[7], Michal Holčapek[8], Harald Köfeler[9], Todd W. Mitchell[10], Markus Wenk[11] and Kim Ekroos[12]

[1] Institute of Clinical Chemistry and Laboratory Medicine, University of Regensburg, Germany.

[2] Leibniz-Institut für Analytische Wissenschaften-ISAS-e.V., Dortmund, Germany

[3] Laboratory for Metabolomics, RIKEN Center for Integrative Medical Sciences (IMS), Tsurumi, Yokohama, Kanagawa 230-0045, Japan

[4] RIKEN Center for Sustainable Resource Science, Yokohama, Japan, National Institute of Genetics, Mishima, Japan

[5] Center for Environmental and Human Toxicology, Department of Physiological Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL, USA

[6] Department of Biochemistry and Molecular Biology, VILLUM Center for Bioanalytical Sciences, University of Southern Denmark, DK-5230 Odense, Denmark.

[7] Swansea University Medical School, ILS1 Building, Singleton Park, Swansea, UK.

[8] Department of Analytical Chemistry, Faculty of Chemical Technology, University of Pardubice, Pardubice, Czech Republic.

[9] Core Facility Mass Spectrometry and Lipidomics, ZMF, Medical University of Graz, A-8010 Graz, Austria

[10] School of Medicine, Illawarra Health and Medical Research Institute, University of Wollongong, Wollongong, NSW 2522, Australia

[11] Singapore Lipidomics Incubator (SLING), Department of Biochemistry, YLL School of Medicine, National University of Singapore, Singapore, Singapore.

[12] Lipidomics Consulting Ltd., FI-02230 Esbo, Finland.

[*]**Corresponding authors:** Gerhard Liebisch; Tel.: +49 941 944 6240, E-mail: Gerhard.Liebisch@ukr.de, and Kim Ekroos; Tel.: +358 40 744 8997, E-mail: kim@lipidomicsconsulting.com.