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Supplementary information S1: Studying sphingolipids: limitations and technical advancements.

The study of sphingolipids presents several obstacles emanating from the lipophilic character of these molecules, the large number of enzymes involved in their metabolism, and the presence of many of their targets and regulators.

In vitro studies (e.g. on enzymes, lipid interaction with target proteins, model membranes) are complicated by the poor water solubility of most lipids. Cellular studies are equally difficult due to limited abilities to deliver bioactive lipids to cells and even less so to specific target compartments and limitations in monitoring in real time fluxes in lipids and changes in their location. These lipid-specific complexities are addressed in more detail in (1).

Nevertheless, there have been several advances in the study of bioactive lipids.

1) Owing to molecular approaches, all key enzymes of sphingolipid metabolism have been identified. In turn, this has enabled an '*enzyme centric*' approach to defining the functions of specific enzymes of the sphingolipid pathway through loss or gain of function studies, as well as biochemical studies on specific enzymes.
2) A large number of model organisms that allow the study of specific sphingolipid pathways through genetic modulation of the relevant sphingolipid enzymes are now available. These include yeast, mice, *Caenorhabditis elegans*, *Drosophila melanogaster*, and plants, especially *Arabidopsis thaliana*.
3) Methodologies such as liquid chromatography–mass spectrometry (LC/MS) assays continue to evolve to determine the molecular structures of various sphingolipids, to quantitate them, and to study their metabolism and regulation. This has enabled a '*lipid centric*' approach to probing sphingolipid function.
4) A systems biology approach is evolving that evaluates sphingolipid metabolism as a network that connects many bioactive sphingolipids such that the metabolism of one is metabolically coupled to additional downstream sphingolipid metabolites. We have termed these as 'metabolic ripple effects' since the action of any one enzyme (e.g. a sphingomyelinase) cannot be *a priori* attributed to its direct product, in this case ceramide, as this ceramide can be further metabolized to sphingosine or

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ceramide 1-phosphate (or other metabolites). Therefore, at a semi comprehensive level, it is often useful to approach bioactive sphingolipids as a network of metabolically connected molecules.

5) Very recent studies are ushering advanced imaging approaches to the study of sphingolipids. This is being accomplished with matrix-assisted laser desorption/ionization (MALDI) imaging. Also, the development of a ^{11}C PET tracer for positron emission tomography (PET) to image the sphingosine-1-phosphate receptor 2 (S1PR2) provides a novel approach to targeted *in vivo* imaging (2).

References

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