Lipid profiling and lipidomics

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Related nutrients/biomarkers: essential fatty acids, infant nutrition biomarkers, energy homeostasis biomarkers.

Importance of lipids for health

Lipids have been implicated in the early onset of several non-communicable and neurodegenerative diseases. Inadequate intake of essential fatty acids, especially during the pregnancy and early life can lead to a reduced cognitive development. Furthermore, many pathologies that have an inflammatory component usually show a change in lipid profiles. Lipidomics brings together developments in mass spectrometry, analytical chemistry and data processing to obtain a rapid overview of the lipid composition of a biological sample.

Lipid metabolism has a very high degree of plasticity and although humans are not able to synthesise a range of fatty acids, alternative pathways can be deployed to maintain homeostasis. Inadequate intake of essential fatty acids, and a deficiency in other nutrients that are crucial in lipid metabolism, such as choline, can lead to inability to produce phospholipids that are essential to produce lipoprotein particles, which will decrease the body's ability to bring lipids into the circulation and therefore lead accumulate in the liver and other organs.

On the other hand**,** poor diet quality can lead to an imbalance in fatty acid intake, especially cheap processed food often use palm oil, rich in palmitic acid (FA(16:0). This can lead to an overexposure to saturated fatty acids, leading to cardiovascular risks and an increase in Low density lipoprotein particles (LDL). A diet rich in saturated fat is often also rich refined carbohydrates and overexposure to these sugars will stimulate the liver to convert these into saturated fat and in the same way alcohol will lead to the same effect. In most cases increased saturated lipids in the circulation are a marker of this de-novo lipogenesis.

Dehydrogenated fats can also be rich in trans fatty acids. The excess intake of these fatty acids has been associated with a range of disease, mainly to an increase of LDL and reduction of HDL leading to higher heart disease risk. This has led to the regulation of the trans fatty acid content of food in many countries.

Background to lipid profiling and lipidomics

Lipidomics brings together developments in mass spectrometry, analytical chemistry and data processing to obtain a rapid overview of the lipid composition of a biological sample. Lipid profiling can be performed on a range of samples (1). Samples can be taken from the circulation (serum, plasma, dried blood spots), tissue or be in vitro materials (1,2). The approach can be fully quantitative, using isotopically-labelled internal standards and calibration curves of specific lipids, to semi-quantitative, or to qualitative approaches (3,4). The method applied needs to be aligned with the research questions and the hypotheses being tested. It is possible to focus the method on a wide range of intact lipids or the method can be focussed on only the fatty acid composition within a certain lipid class or sample type.

With the right approach, it is possible to determine the deficiencies in essential fatty acids, such as n3-poly unsaturated fatty acids (n3-PUFA) (5), or over exposure to unhealthy fatty acids, such trans-fatty acids from hydrogenated fats (6). It is also possible to use lipid profiling of dried blood spots from heel prick samples to determine if infants were either breastfed or formula fed (7). Different studies have showed that specific lipid profiles in specific sample types are either diagnostic or prognostic of specific or general pathologies (1). For instance, the lipid profile of a plasma sample of early pregnancy (week 12-17) can be predictive of gestational diabetes (8). The lipid profile has been implicated in the early onset of deregulation of energy homeostasis (e.g. insulin resistance) (1), liver pathologies (e.g. nonalcoholic fatty liver disease, NAFLD) (9) and neurodegenerative disease (e.g. Alzheimer's disease) (10). Many pathologies that have an inflammatory component usually show a change in arachidonic acid. It is possible to follow this in more detail through the analysis of lipid mediators (metabolites from arachidonic acid and n-3 PUFAs) although the active compounds have a very short half-life and are only active locally, therefore demanding very careful sample collection and preparation.

The quality of the results obtained by lipidomics approaches is highly dependent on the quality of samples. Sample collection, storage and processing can all have a profound effect on the lipid measurements. It has been shown that reproducible data can be obtained in large scale cohort

studies, although this is highly dependent on the quality control of the methods and the obtained data.

Reported lipid biomarkers

Many studies have determined the association between specific lipids and specific pathologies, nutrients or diets. This does not mean that these lipids are biomarkers.

Table 1 gives an overview of associations between lipids and nutrition that have been reproduced by different studies, either using independent methods or independent cohorts.

Table 1: Lipid/fatty acid biomarkers for recent intake and status modified from (1)

Biomarker	Analysis type	Sample	Diet
Ratio PC(36:2)/SM(39:1)	LCMS and DIMS	Dried blood spots	Marker of breast milk consumption (7)
FA(22:6n-3) FA(20:5n3)	GC	Serum, Plasma, whole blood	Marine derived fats (11)
CE(22:6), CE(20:5), PC(36:5), PC(38:6)	LCMS and DIMS	Serum, Plasma, whole blood	Marine derived fats (fish oil, fatty fish etc) (5)
FA(15:0), FA(23:0)	GC	Serum, Plasma, whole blood	Dairy fat (12)
PC(35:1), SM(39:1)	LCMS and DIMS	Serum, Plasma, whole blood	Dairy fat (12)

Abbreviations: PC(36:2): phosphatidylcholine(36:2); SM(39:1): sphingomyelin(39:1); FA(22:6n3): fatty acid (22:6n3) or DHA; FA(20:5n3) fatty acid (20:5n3) or EPA; CE(22:6): Cholesteryl ester (22:6), CE(20:5): Cholesteryl ester (20:5); PC(36:5): phosphatidylcholine (36:5); PC(38:6): phosphatidylcholine(36:5); FA(15:0): fatty acid (15:0); FA(23:0): fatty acid (23:0); PC(35:1): phosphatidylcholine(35:1); SM(39:1): sphingomyelin(39:1); LCMS: Liquid chromatography hyphenated with mass spectrometry; DIMS: direct infusion mass spectrometry; GC: Gas chromatography

Methods of analysis

Direct infusion mass spectrometry (DIMS)

Organic extracts are infused into a high-resolution mass spectrometer using chip-based nanospray. Data can be collected in positive mode or in negative mode or both. Data processing protocols are necessary to deconvolute the spectra and obtain measurements per lipid signal. Internal standards can be used to quantify the results.

Liquid chromatography hyphenated with mass spectrometry (LCMS)

Instead of direct infusion, it is possible to separate the lipids first by chromatography, most approaches will use a reversed phase column material, that will retain lipids by their polarity (retaining more apolar lipids). The mass spectrometer can by based on high resolution or on specific fragmentations (triple quadrupole). Each lipid will have a specific retention time and mass spectral feature that will be detected. Again, internal standards can be used to enable quantification.

Gas Chromatography

It is possible to measure the individual fatty acids which are with a lipid fraction (e.g. phospholipids) or within the whole sample. The standard method is to hydrolyse the extracts and derivatise the fatty acids into their methyl esters (FAMEs). These can be separated by Gas chromatography and both a flame ionisation detector or an electron impact mass spectrometer can be used to for identification and quantification.

Quality control and technical assistance

Quality Control

There are no international accreditation schemes for lipidomics but the National Institute of Standards and Technology produces Standard Reference Material (SRM) 1950 for which quantified lipid concentration are available.

Technical assistance

For technical assistance and questions on lipids, lipid profiling and lipidomics, please contact open-global via https://open-global.kcl.ac.uk/contact/ or write to:

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Website: http://www.mrc-epid.cam.ac.uk/people/albert-koulman/
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Useful links

cMaLL combined lipidomics training and sample analysis course: for more information, see the OpeN-Global page on training opportunities, or link directly to the course information.

https://open-global.kcl.ac.uk/training-opportunities/

https://open-global.kcl.ac.uk/cmall/

Further reading

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