Lecture 11. Lypidomics.

Learning outcomes:
1. Give the definition to the terms “lipids”, “lypoproteins”, “lypidome”, “lypidomics”.
2. Analyze the different types of lypids by their chemical structure and function, give the specific examples.
3. Explain the methods of lypidomic research.
4. Explain different disturbances of lypid metabolism and methods of their diagnostics and treatment, give the specific examples.

Lipidomics is the large-scale study of pathways and networks of cellular lipids in biological systems. The word "**lipidome**" is used to describe the complete lipid profile within a cell, tissue, organism, or ecosystem and is a subset of the "**metabolome**" which also includes the three other major classes of biological molecules: proteins/amino-acids, sugars and nucleic acids. Lipidomics is a relatively recent research field that has been driven by rapid advances in technologies such as **mass spectrometry (MS)**, **nuclear magnetic resonance (NMR) spectroscopy, fluorescence spectroscopy, dual polarisation interferometry** and **computational methods**, coupled with the recognition of the role of lipids in many metabolic diseases such as obesity, atherosclerosis, stroke, hypertension and diabetes. This rapidly expanding field complements the huge progress made in genomics and proteomics, all of which constitute the family of systems biology.
Lipids are a diverse and ubiquitous group of compounds which have many key biological functions, such as acting as structural components of cell membranes, serving as energy storage sources and participating in signaling pathways. Lipids may be broadly defined as **hydrophobic** or **amphipathic** small molecules that originate entirely or in part from two distinct types of biochemical subunits or "building blocks": ketoacyl and isoprene groups. The huge structural diversity found in lipids arises from the biosynthesis of various combinations of these building blocks. For example, **glycerophospholipids** are composed of a glycerol backbone linked to one of approximately 10 possible headgroups and also to 2 fatty acyl/alkyl chains, which in turn may have 30 or more different molecular structures. In practice, not all possible permutations are detected experimentally, due to chain preferences depending on the cell type and also to detection limits - nevertheless several hundred distinct glycerophospholipid molecular species have been detected in mammalian cells. **Plant chloroplast thylakoid membranes** however, have unique lipid composition as they are deficient in phospholipids. Also, their largest constituent, **monogalactosyl diglyceride or MGDG**, does not form aqueous bilayers. Nevertheless, dynamic studies reveal a normal lipid bilayer organisation in thylakoid membranes.
Most methods of lipid **extraction** and **isolation** from biological samples exploit the **high solubility of hydrocarbon chains in organic solvents**. Given the diversity in lipid classes, it is not possible to accommodate all classes with a common extraction method. The traditional Bligh/Dyer procedure uses **chloroform/methanol**-based protocols that include phase partitioning into the organic layer. These protocols work relatively well for a wide variety of physiologically relevant lipids but they have to be adapted for complex lipid chemistries and low-abundance and labile lipid metabolites. When organic soil was used, **citrate buffer** in the extraction mixture gave higher amounts of lipid phosphate than **acetate buffer, Tris, H2O or phosphate buffer**. The simplest method of **lipid separation** is the use **of thin layer chromatography (TLC)**. Although not as sensitive as other methods of lipid detection, it offers a rapid and comprehensive screening tool prior to more sensitive and sophisticated techniques. **Solid-phase extraction (SPE) chromatography** is useful for rapid, preparative separation of crude lipid mixtures into different lipid classes. This involves the use of prepacked columns containing silica or other stationary phases to separate glycerophospholipids, fatty acids, cholesteryl esters, glycerolipids, and sterols from crude lipid mixtures. **High-performance**

liquid chromatography (HPLC or LC) is extensively used in lipidomic analysis to separate lipids prior to mass analysis. Separation can be achieved by either **normal-phase (NP) HPLC or reverse-phase (RP) HPLC**. For example, NP-HPLC effectively separates glycerophospholipids on the basis of headgroup polarity, whereas RP-HPLC effectively separates fatty acids such as eicosanoids on the basis of chain length, degree of unsaturation and substitution. For global, untargeted lipidomic studies it is common to use both RP and NP or **Hydrophilic Interaction Liquid Chromatrography (HILC)** columns for increased lipidome coverage. The application of **nano-flow liquid chromatography (nLC)** proved thereby to be most efficient to enhance both general measurement sensitivity and lipidome coverage for a global lipidomics approach. **Chromatographic (HPLC/UHPLC) separation** of lipids may either be performed offline or online where the eluate is integrated with the ionization source of a **mass spectrometer**. The progress of modern lipidomics has been greatly accelerated by the development of **spectrometric methods** in general and soft ionization techniques for mass spectrometry such as **electrospray ionization (ESI)**, **desorption electrospray ionization (DESI)**, and **matrix-assisted laser desorption/ionization (MALDI)** in particular. "Soft" ionization does not cause extensive fragmentation, so that comprehensive detection of an entire range of lipids within a complex mixture can be correlated to experimental conditions or disease state. In addition, the technique of **atmospheric pressure chemical ionization (APCI)** has become increasingly popular for the analysis of nonpolar lipids.

The questions for self - control:

1. What are the “lipids”, “lypoproteins”, “lypidome”, “lypidomics”?
2. Chemical structure and functions of the different types of lypids.
3. Methods of lypidomic research.
4. Diseases connected with the disturbances of lypid metabolism and methods of their diagnostics and treatment.

Recommended readings:

1. Wenk MR (July 2005). "The emerging field of lipidomics". Nat Rev Drug Discov. 4 (7): 594–610. doi:10.1038/nrd1776. PMID 16052242. S2CID 83931214.
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