Journal of Proteomics xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Journal of Proteomics

journal homepage: www.elsevier.com/locate/jprot

Review

# Gender-related metabolomics and lipidomics: From experimental animal models to clinical evidence

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ARTICLE INFO

*Keywords:*
Metabolome
Lipidome
Sex
Biomarkers

ABSTRACT

Lipidomics and metabolomics have emerged as important tools for the characterization of specific physiological and pathological traits. The selection of the analytical approaches and the choice of a targeted rather than an untargeted strategy in metabolomics find their reasons in the driving hypothesis of the study, sample features and instrumental availability. Moreover, in the last years, −omics approaches have found their application in the study of sex-related dimorphism. In this review, lipidomic and metabolomic analyses are presented in a biomedical perspective. Here, we provide an updated overview covering recent applications of metabolomics and lipidomics in the area of sex-related differences in human and preclinical models. Experimental evidence underlines that sex is one of the most relevant biological variables significantly influencing metabolomic and lipidomic profiles. This knowledge can be exploited for the identification of novel sex-specific biomarkers and innovative targets relevant for gender medicine.

## 1. Introduction

In the last years, research in the field of life sciences has been focusing on the holistic view of the molecular species that participate in all cellular processes, i.e. genes, transcripts, proteins, and metabolites. Due to the current technological means, scientists have now the possibility to perform large-scale and comprehensive analyses named “omics” that all together contribute to gain an integrated view of biological systems, thus providing experimental basis to systems biology models. The basic idea of this approach is that biological processes can be analyzed in detail if considered as a vast net of interactions, all parts of a whole system [1]. In most cases, achievements in the field of life sciences have been obtained by means of hypothesis-driven experiments that lead to specific, although circumscribed conclusions. On the other hand, omics analyses are not driven by a specific hypothesis but are performed to answer to a more general scientific question in an unbiased fashion leading to many different hypotheses and unexpected perspectives.

Omics technologies find their application in different fields such as biomedical [2], agricultural [3], environmental [4] and nutritional sciences [5]. In life sciences, they are now widely used in preclinical research for the identification and the characterization of undescribed molecular mechanisms. In clinical medicine they represent a useful tool for the evaluation and the characterization of specific features in a given population, for the screening of unknown biomarkers, and for drug discovery and validation [6]. In addition, the integration of multi-omics technologies by bioinformatic tools has emerged as an original approach. Merging of multiple disciplines may help the characterization of genome-phenotype relationship, where the understanding of causal interactions between genome, transcriptome, proteome and metabolome will increase our chances to understand the great complexity of biological systems (Fig. 1).

Metabolites are small molecules and intermediates of metabolism, which participate to various cellular functions and processes, from energy metabolism to modulation of protein activity, signaling pathways and defense mechanisms. Metabolites can be classified depending on the metabolic pathway they belong to or according to their physicochemical properties. In respect to this latter feature, lipids are defined as fat-soluble compounds based on their solubility in non-polar solvents and show an enormous molecular diversity. Lipids are indispensable molecules for cells, as they represent the main component of biological membranes, a great energy storage, and important substrates for post-translational modifications [7]. In addition, they may also function as an important class of intracellular and extracellular signals [8]. In the last two decades we have seen an exponential growth of papers reporting metabolomic and lipidomic analyses, mainly conducted by liquid chromatography-coupled mass spectrometry (LC-MS/MS), applied to different fields of life sciences (Fig. 2). This burst has occurred due to technological advancements that have allowed:

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https://doi.org/10.1016/j.jprot.2017.11.001
Received 2 August 2017; Received in revised form 16 October 2017; Accepted 1 November 2017

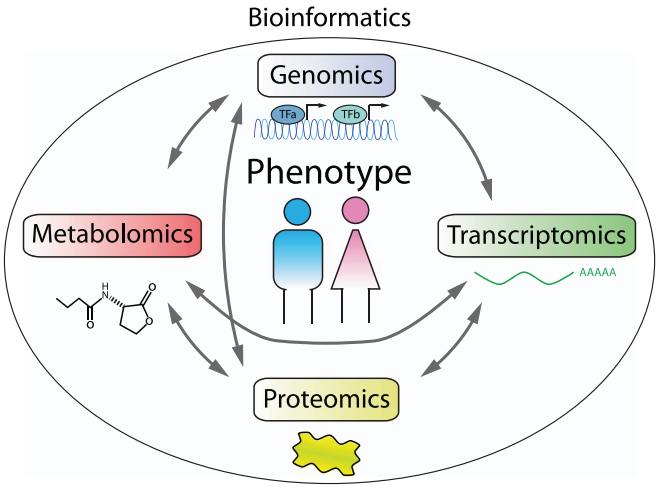


**Fig. 1.** Omics approaches aim to analyze a set of molecules and their integration represent a powerful framework for studying specific phenotypic traits. The first omics being applied were genomics and transcriptomics, followed by proteomics and metabolomics. The integration of these disciplines raised the necessity to develop sophisticated bioinformatic tool able to combine information from different -omic analyses.

i) analysis of multiple metabolites in a single run, thus simplifying operations and reducing machine time;
ii) development of dedicated software for omic-data handling and possible integration with data sets obtained by means of other omic approaches (i.e. transcriptomics, proteomics).

Based on these premises, the aim of the present review is to provide an updated survey of metabolomics and lipidomics, with a specific focus on their application in the understanding of sex-related metabolic features.

## 2. Metabolomic and lipidomic workflows: from sample preparation to statistical analysis

Currently, the main strategies used for metabolomics approaches are “untargeted metabolomics” (or metabolic fingerprinting) and “targeted metabolomics” (or metabolic profiling). In the first case, the approach aims at identifying the greatest number of metabolites in a given sample in a single run. On the other hand, a targeted approach is focused on the analysis of preselected metabolites, and, particularly, it is widely used to obtain quantitative data of specific compounds [5].

The key task in a metabolomic experiment is represented by sample collection and preparation for the analysis. Sample collection is of outmost importance given the final goal of the experiment. Indeed, metabolome of a frozen tissue can be very different from that obtained in a freshly collected specimen due to degradation of some metabolites. Furthermore, also the post-collection freezing delay time is another parameter that should be careful standardized because it may affect the final metabolic profile [9]. The optimization of sample extraction procedures ensures experimental reproducibility and the best quality of the analysis. Therefore, the extraction method should be chosen depending on the type of metabolomic approach to be subsequently applied, i.e. metabolic fingerprinting or metabolic profiling. Indeed, in “untargeted metabolomics” a minimal or no sample preparation should be employed to avoid loss of metabolites from the biological samples. On the other hand, by choosing a “targeted approach”, an oriented extraction and purification method of selected metabolites should be carried out [5]. In addition, the nature of metabolites should be considered since metabolites are chemically diverse. They can be grouped in water-soluble or hydrophilic compounds and in water-insoluble or hydrophobic compounds, requiring different extraction procedures.

Several works report a simple procedure to extract lipids and hydrophilic metabolites at the same time. These protocols separate and recover the water soluble and lipid metabolite fractions by adding to samples a mixture of chloroform:methanol (1:1, v/v) or chloroform:-water (2:1, v/v), followed by vortex-mix and then centrifugation. Thus, a phase containing polar metabolites (upper phase) and a phase containing lipids (bottom phase) are obtained [10,11].

In the case of a lipid-oriented extraction, several different mixture of organic solvents could be used, however one of the most common is the Folch extraction method [12] with some modification, i.e., chloroform:methanol (2:1, v/v) or methyl tert-butyl ether (MTBE)-methanol-water or MTBE-methanol-chloroform [10,13].

Lipid species are differently abundant in biofluids (plasma, serum, urine, etc.) or in tissues and can be also largely different within the biofluid and/or the tissue considered. Based on our experience and on that reported by other investigators, 5–100 μL of biofluids or 1–100 mg of tissues represents an adequate range of material for lipidomic analyses [14,15].

In general, a sample pre-treatment step, such as solid phase extraction (SPE) or liquid-liquid extraction, could be performed before running the chromatographic separation and/or data acquisition [16].

The following step is the data acquisition: many analytical methods have been developed to retrieve useful information from biological systems. One of the main aims in the field of -omics is to process relatively large set of samples and related data in the shortest time with little or no sample preparation. The development of rapid and

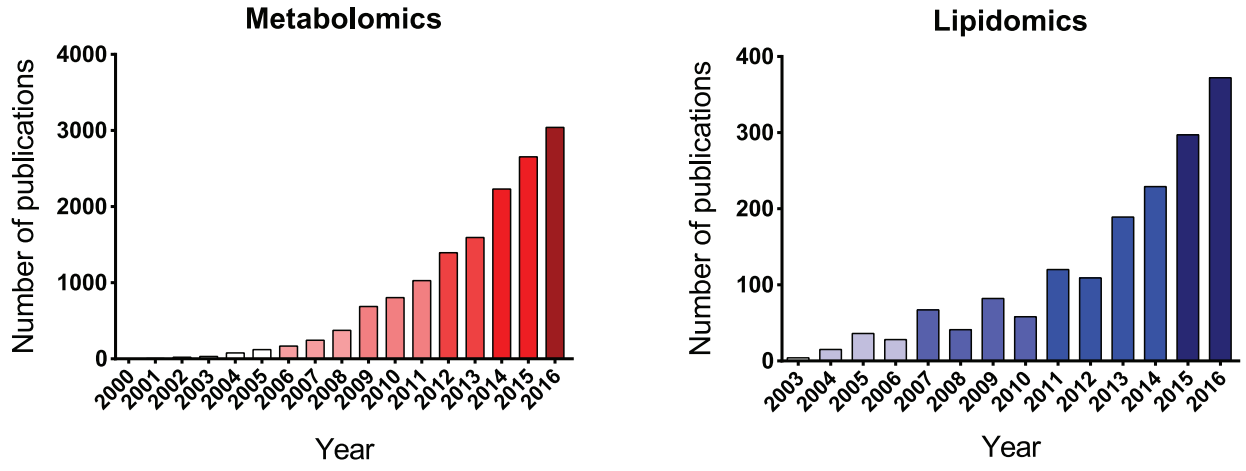


**Fig. 2.** Metabolomics and lipidomics publication metrics from 2000 through 2016. The bar graph shows the number of publications that list a key word (metabolomics and lipidomics) derived from the search in PubMed database.