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Scope of the Thesis

Over the last decades omics technologies have helped us to fundamentally change how biomedical research is carried out. The general key and most attractive idea is the simultaneous monitoring of hundreds or thousands of macro- and small molecules to subsequently observe multiple (or perhaps all) cellular pathways. This new paradigm in molecular biology is allowing us a rapid increase in understanding previously unknown cellular molecular details and their relationships with tissue functions.

At the end of the last century we witnessed the genomics revolution, followed by important advancements in proteomics and transcriptomics technologies. Nowadays, mapping the complete set of metabolites present in a biological sample, also known as metabolomics, constitutes the next step in the evolution of the omics field. Due to its focus on the downstream output of biochemical networks, metabolomics is considered to reveal the last part of the "omics spectrum" and therefore to be the closest representation of a cellular or organismal phenotype. Lipids are a subset of the metabolome representing 70% of the entries in the Human Metabolome Database (HMDB). It is thus no surprise that lipidomics is currently considered a separate discipline besides metabolomics. However, from an experimental point of view it is important to highlight that both are subjected to rather similar challenges.

Despite their rapid growth, metabolomics and lipidomics are still in their infancy. As such, the work described in this thesis tries to tackle different aspects and common drawbacks yet to be comprehensively addressed during the development of metabolomics/lipidomics studies, including experimental issues at the pre-analytical stage (**Chapters 2** to **5**) and a translational work describing the usefulness of a metabolite as a possible biomarker for one of the most widespread human pathophysiologies (**Chapters 6** and **7**).

Chapter 2 presents an extensive overview of the scientific literature on non-enzymatic energy metabolite degradation/interconversion chemistry in metabolomics studies of the central carbon metabolism. Specifically, the chapter focusses on qualitative as well as quantitative aspects that may affect the acquisition of accurate data in the context of metabolomics studies, and provides an experimental example of the issues of using isotopically labeled internal standards in such studies.

Chapter 3 explores the stability of nucleotide triphosphates under common experimental conditions of the boiling ethanol extraction method, a frequently used approach for metabolomics studies of biological samples. We further study how a complex cellular matrix extracted from yeast (*S. cerevisiae*) affects the degradation profiles and which are the implications of its use for quantitative metabolomics studies.

Chapter 4 provides an overview of lipidomics, a derivation of metabolomics and the most recent member of the omics technologies. There is a lack of awareness on how lipid stability is affected during the pre-analytical stage (from the experimental design to lipid extraction). Thus, at this point we focus on common pitfalls during a typical lipidomics workflow and suggest ways to avoid them.

Chapter 5 focuses on extraction as a critical step of the pre-analytical stage for lipid analysis. Here, we used an untargeted lipidomics approach to compare the differences/similarities between the most commonly used two-phase extraction systems and a recently introduced one-phase extraction system for lipid analysis. We also describe a novel approach to quantify relationships between different extraction methods and a pooled sample by using hierarchical clustering analysis (HCA).

Chapter 6 presents a translational work in which we establish the strong involvement of *Oplah,* a gene encoding for 5-oxoprolinase, in the development of heart failure (HF). We then proved that cardiac injury leads to OPLAH depletion and subsequently an increase in 5-oxoproline levels, the substrate of OPLAH, and oxidative stress, while OPLAH overexpression improved cardiac function after ischemic injury. Finally, we observed in patients with HF that elevated plasma 5-oxoproline levels are associated with a worse clinical outcome. This translational approach provides important insights onto the usefulness of 5-oxoproline as a putative circulating marker for predicting adverse outcome in patients with HF and proposes OPLAH as a potential target for therapeutic intervention.

Chapter 7 describes the development and validation of an LC-MS method to measure 5-oxoproline, L-glutamate, GSH and GSSG. These metabolites are key components of the γ-glutamyl cycle that were quantitatively evaluated to study the effect of heart failure in different biological samples from mice (heart, kidney and liver tissue, as well as plasma and urine). We determine that besides the ratio GSH/GSSG ratio, 5-oxoproline may also serve as an easily measurable marker for oxidative stress resulting from cardiac injury.

Chapter 8 gives an overview of all findings in this thesis, as well as some final remarks and future perspectives of the importance of evaluating pre-analytical factors in metabolomics and lipidomics research.