Systems biology for evaluating system-based medicine

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Chapter 7

Conclusion remarks and perspectives

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Obesity and diabetes are rapidly becoming an epidemic threat to health care affecting more than 120 million people worldwide. In the early stages of disease development these disorders are part of a condition called metabolic syndrome. One of the features involved in metabolic syndrome is related to atherogenic dyslipidemia and consequently lipid-lowering therapies for treating subjects with metabolic syndrome are of great importance in today's strategies for improvement of global health care.

However, living organisms often respond in complex and unpredictable ways to inner or outer stimuli causing a disease state with dynamic changes in the level of multiple metabolites as present in complex biological fluids and tissues. As a consequence, a comprehensive approach to investigate organisms as an integrative system of interacting genes, proteins and metabolites (*i.e.* systems biology) is fundamental to unravelling interactions of complex pathophysiological mechanisms in organisms. In practice, animal models are often used to study dietary implications. Animal models obtained via gene manipulation are usually developed for studying risk factors associated with the prevalence of life-style related human diseases via an integrated study of genes, proteins and metabolites in the entire system. When a multi-factorial experimental design is used, systems biology can offer new ways to improve the quality of human life and provide in-depth insights into health and disease.

The studies described in this thesis reveal that systematic analytical strategies focusing on measuring a large set of small-molecular weight metabolites in body fluids, tissues and/or whole organs are very promising in finding potential biomarkers for disease prevention/diagnosis, health promotion or (combination) drug (herbal) treatment when combined with appropriate bioinformatics tools. Such holistic approaches were used for comprehensive analysis of lipid profiles, so-called lipidomics, to evaluate the treatment effects of a single chemical drug and of complex herbal medicine on changes in endogenous lipid metabolites in plasma and/or liver tissues.

As lipids are fundamental components of biological membranes, they are likely to reflect, directly or indirectly, the interaction with molecular targets in cells, tissues, and/or organs. Monitoring the subtle changes of lipids caused by genetics, risk factors, and exposure to agents quantitatively and qualitatively is key to understanding the pathophysiological role of lipids in development of disease. It is furthermore helpful for establishing preventive approaches for health care. However, lipidomic analysis encounters many challenges due to the complexity of lipids, the diversity of biological samples and the limitation of analytical technologies (reviewed in **Chapter 2**). To overcome these challenges, advanced techniques with high mass accuracy and high mass resolution (*i.e.* linear ion trap-Fourier transfer ion cyclotron mass spectrometry − FTMS) were used in this thesis and were the basis to develop a novel method for the profiling of numerous lipids in plasma (**Chapter 3**) and liver (**Chapter 5**) samples. Different columns were compared and the chromatographic conditions were optimized to get better separation. In total, more than 160 individual lipid molecular species from 8 different classes were detected in a single measurement within 30 min. The

outcome of the method validation demonstrates that the developed lipidomics platform is simple, fast and reliable for routinely determining lipid molecular species that span from polar to apolar.

In this thesis, the integration of analytical chemistry, bioinformatics and lipid biochemistry was successfully applied in our lipidomics studies. In one study, a combined approach was used that involved applying RPLC−MS-based lipidomics for measuring biochemical parameters and profiling of the constituents of ginseng roots of different ages. Multi-variate statistics was used to predict the root age dependent bioactivity of ginseng in the treatment of diabetes (**Chapter 4**). The outcome of the study reveals that this well-known multi-component herb showed root age dependent therapeutic effects on hyperlipidemia and hyperglycemia in type II diabetic Goto-Kakizaki rats. These root age dependent biological effects may be linked with the variation in both the ratios and concentration of specific bioactive ginsenosides in ginseng roots of different growth ages. It shows that bioactive synergetic components can be identified in this manner. These results provide opportunities for developing a new class of agents for diabetes care. A similar strategy was successfully applied to two other studies in which changes in lipid profiles of the ApoE*3Leiden.CETP mice with induced early stage of diabetes were detected after exposure to single chemical drug – rimonabant (**Chapter 5**) and a multi-preparation Chinese medicine – SUB885C (Chapter 6), respectively. The outcome shows that anti-atherogenic changes in lipids of mice with early obesity caused by SUB885C have similarities with effects caused by known drugs (*e.g.* rimonabant, atorvastatin, niacin). These studies demonstrate that complex herbal medicines offer opportunities in treatment of metabolomic syndrome in an early phase and amelioration of the early symptoms. The above-mentioned approach based on systems-biology is very promising in unraveling effects of interventions on lipid metabolism and is a useful tool to bridge Chinese medicine and Western medicine at the biochemical level.

Although many studies reveal that lipidomics is a powerful tool for diagnosing disorders related to lipid metabolism, the lack of standardization for assaying lipids in biofluids or tissues makes comparison of results of different studies difficult and sometimes impossible. The ideal way to solve this issue is to provide quantitative data for each individual lipid species in a given sample. However, it is very difficult to achieve this goal with the current lipidomics approaches. This is due to the fact that it is not possible to get a large set of exogenous or labelled endogenous reference lipids that could be used as internal standards, both from the availability and the economical point of view. In practice it is therefore more attractive to use standards aiming at groups of lipids with similar chemical properties. Accordingly, lipidomics investigations studying a defined number of specific lipid metabolites (*i.e.* targeted lipidomics) in a quantitative manner will play an important role in finding potential biomarkers, new targets or ingredients for lifestyle-related metabolic abnormality.

Another important issue in lipidomics is elucidation of the structure of lipids. The diversity and large concentration ranges of lipids make identification of the structure of lipids detected with the current LC-MS-based lipidomics platforms difficult. Often low abundant lipid species play an important role in pathopysiological processes in biological systems and the challenge in identification of low abundant lipids should not be underestimated. In this

thesis, this was mainly overcome by using FT–MS. Moreover, most lipid molecules especially those belonging to the same lipid class produce MS/MS spectra with characteristic ions, which enables the identification of lipids in many cases. It should be mentioned that identification of lipids can be achieved at different levels, from the elemental composition, to the identification of the elemental composition of the fatty acid group at the different positions of the glycerol backbone, up to the position of the double bonds and even their conformation (cis/trans) of the acyl group at the *sn*-1 and *sn*-2 position.

Like the other 'omics' sciences, lipidomic studies generate vast amount of data.. The benefit of lipidomics to answer biological questions will only be realized when the data produced are analyzed with appropriate biostatistical and bioinformatic tools to reveal hidden correlations, determining statistical significance and detecting trends in datasets across samples. Multivariate data analysis proved to be mandatory in classifying samples for anti-diabetic treatments (**Chapter 4**) and anti-obesity effects (**Chapter 5** and **Chapter 6**). However, the datasets obtained by lipidomics are in most cases confined to very limited number of samples as compared to the number of variables. Therefore validation of not only the analytical method but also of the univariate and multivariate data analysis methods is very important and should be done with great care.

In the past decade biomarker research by lipidomics has been growing continuously. Successful application areas for these studies have been in the domain of nutrition studies, cancer research, and in particular the diagnosis and treatment of lipid metabolic diseases. Until now, the majority of these studies is very descriptive and rarely supports the lipidomic findings as observed with experimental or clinical studies related to the function of distinct lipid biomarkers. Specifically, interesting results of non-targeted lipidomics approaches often lack an explanation concerning the specific function of detected lipid biomarkers or lipid metabolite patterns in the investigated physiological or disease-related context. This can be attributed to the often not fully identified structure of the lipids, the fact that only recently detailed information on the lipid composition is available and many details of the biochemistry of lipids in the different organs and compartments is still not known. A better biological interpretation of lipidomics study requires a close and efficient interplay between biochemical/medical and analytical chemical research scientists. Therefore, close collaboration of scientific experts in different disciplines is absolutely mandatory to unravel lipid-related functional mechanisms of biomarkers detected and to obtain more in-depth knowledge of lipid-related biological relationships (*e.g.* pathways, networks and regulation mechanisms) concerning the action of single chemical drug (herbs) on subjects or characterization of a disease.

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