Summary

The introduction of systems biology in combination with the profiling of numerous biochemical components (*e.g.* lipid metabolites, herbal products) enables the study of living systems from a holistic perspective. Such a system-based approach has opened up a unique opportunity to understand biochemical events taking place in healthy or diseased organisms. In this thesis we explored systems biology-based platforms to investigate the therapeutic effects of chemical drugs and herbal medicines on animal models with high-fat diet-induced obesity and genetic manipulated diabetes. The aim of the work was to better understand the working mechanisms of both treatments on metabolic syndrome from a holistic point of view and to evaluate the potentials of 'omics' technologies to this effort.

In **Chapter 2** the challenges in comprehensive analysis of lipids in complex biological samples such as body fluids, cell lines and tissues were discussed. In addition, the present status of analytical strategies in lipidomics and their representative applications in biomarker discovery related to disease or therapeutical intervention were thoroughly reviewed. Even so, a particular example of multivariate statistical analysis of plasma lipidomics data from rats and humans was given to illustrate the powerful capacity of bioinformatics tools in discovery of lipid potential biomarkers in relation to different physiological conditions or phenotypes by investigating disease pathology and intervention effects.

Lipidomics is a lipid-targeted metabolomics approach aiming at comprehensively measuring and mathematically modelling changes in the levels of products of lipid metabolism in biological systems. Currently, it has been a global research hotspot due to its biological significance and the advances in technologies. In **Chapter 3** the development of a novel and fully validated method was discussed for the profiling of lipids in plasma of human and animals. The outcome of analytical characteristics of the method demonstrates that the analytical performance is high-throughput (hundreds of lipids detected in one injection), fast (30 min, the whole running time) and reliable for routinely studying lipids in complex biological samples, manifested as linearity (R^2, 0.994–1.000), limit of detection (0.08–1.28 μg/ml plasma), intra-day repeatability (RSD, 3–8%), inter-day repeatability (RSD, 3–16%), and reproducible recoveries (LPC, 80–85%; PG, 67–71%; PC, 91–105%; PE, 90–100%; TG, 94–105%). It outperforms over many other lipid methods in literature with less sample volume (15–30 μl), shorter analytical time (18 min, lipid separation time) and better lipid separation. In addition, the developed method was successfully applied to study the phenotypic effects of p53 expression in p53 mutant transgenic mice. The capacity of the platform in searching of potential lipid biomarkers in relation to disease prevention and health promotion of subjects under different physiological and pathological status was challenged and evaluated in the subsequent research studies described in this thesis.

In **Chapter 4** the developed lipidomics platform was applied to evaluate 9-week treatment effects of a single chemical compound (*i.e.* metformin) and a well-known multi-component herb (*i.e. Panax ginseng Radix* with different growth age) on the regulation of glycaemic control, lipid-related metabolic parameters and plasma lipid metabolism in Goto-Kakizaki rat

model with spontaneous type 2 diabetes (T2D). A combined approach was used that involved applying liquid chromatography–mass spectrometry (LC-MS)-based lipidomics, measuring biochemical parameters and LC–MS-based profiling of the components of ginseng roots. We hypothesized that the bioactivities of ginseng roots on improving glycemia and lipid metabolism in diabetic GK rats are growth age-dependent. The results from lipid-related plasma metabolomic parameters showed that treatment with 4- and 6-year-old ginseng roots significantly improved glucose disposal, and 5-year-old ginseng treatment significantly increased high density lipoprotein cholesterol as compared to the untreated controls; treatment with 6-year-old ginseng significantly decreased total plasma TG and VLDL-C and improved plasma glycated hemoglobin. In terms of lipidomics, treatments with 4- to 6-year-old ginseng influenced plasma lipid metabolism in diabetic GK rats by reducing TG lipid species. Metformin significantly reduced fasting blood glucose and reduced HbA1c, but showed no effects on plasma lipids. The results demonstrate that ginseng roots show growth age-dependent therapeutic effects on hyperlipidemia and hyperglycemia in diabetic GK rats. These age-dependent effects may be linked with the variation in both the ratios and concentrations of specific bioactive ginsenosides in ginseng roots of different growth ages. Further experiments on the medicinal use of purified individual bioactive compound or combination of specific ginsenosides are necessary to unravel the exact mechanisms of the observed multi-dimensional therapeutic effects of ginseng roots on T2D. These studies are expected to provide an opportunity to develop a new class of agents for diabetes care. This line of research may provide more insight into the quality control of herbal extracts in the development of new bioactive products.

In **Chapter 5** the developed lipidomics platform was applied to characterize and quantify lipids in plasma samples from apolipoprotein E3 Leiden cholesteryl ester transfer protein (ApoE*3Leiden.CETP) transgenic mice on high-fat diet, and to study the differences in lipid compositions between these mice upon a 4-week rimonabant intervention and no treatment controls. Lipid related plasma biochemical parameters and lipoprotein profiles were also investigated to this effort. Rimonabant was found to induce a significant body weight loss, a significant plasma total cholesterol reduction and a clear plasma TG reduction tendency. Principle component analysis (PCA) of plasma lipidomics data set (131 lipid molecular species presented in all mice) revealed distinct lipid clustering patterns between two study groups. Six plasma lipid species were identified to most significantly respond to rimonabant intervention. In addition, hepatic lipidomics of these ApoE*3Leiden.CETP transgenic mice responds to rimonabant treatment was also investigated after an intensive validation of the platform in analysis of liver lipids. Similar to the PCA outcome of plasma lipidomics, a clustering of two studied groups was observed in liver lipidomics data set (133 lipid entries detected in all mice liver). Three lipid species were found to contribute most to the clustering pattern of liver lipids. Finding only limited amount of significant lipid changes in the present study may be attributed to the early stage of obesity in the animal model used. The results from plasma and liver lipidomics indicate that the effects of rimonabant on body weight and cardiovascular risk factors are moderate in the case of early stage of obesity.

Due to the complexity and multi-factorial manifestations of metabolic syndrome, pharmacological strategy with a single chemical compound exhibits limits for addressing a complex regulatory network in abnormal living biosystem. For this reason, combination therapy addressing multiple targets with low or no side effects has become a new field of attention. In **Chapter 6**, multiple pathway effects of a multi-component preparation (so called SUB885C) on plasma and liver lipid metabolism in ApoE*3Leiden.CETP transgenic mice model with early metabolic syndrome were further investigated. We examined (1) the treatment effects of SUB885C on regulation of body weight and lipid-related metabolic parameters, (2) the possible correlation between cholesteryl ester transfer protein and SUB885C induced regulation of lipids and lipoprotein profiles, (3) the activity of SUB885C in cannabinoid-1 receptor binding affinity and lipogenesis. The results showed that SUB885C treatment produced no effect on food intake or body weight, but significantly decreased plasma cholesterol and triglyceride, plasma CETP levels and CETP activity, and significantly increased HDL-C. The significantly increased HDL-C upon SUB885C therapy may be attributed to the decreased CETP level and CETP activity or reduced level of VLDL-TG which would reduce CETP transfer activity by decreasing the transfer of cholesteryl from HDLs to TG–enriched lipoproteins, leading to more cholesterol-enriched HDL particles. In addition, i*n vitro* SUB885C extract was found to cause adipolysis stimulation and adipogenesis inhibition in 3T3-L1 cells. The experiments described in this chapter demonstrate that new preparations at the nutrition-pharma interface can be used to prevent metabolic syndrome or ameliorate its first symptoms. This work reveals that systems biology-based lipidomics approach is very promising in unravelling effects of medical treatment on subjects and useful for finding new targets or ingredients in the field of medication.

In conclusion, the studies described in this thesis showed that the systems biology based lipidomics approach with appropriate bioinformatics tools are essential to describe the global, dynamic metabolic response of living systems, *e.g.* from homeostasis via sub-optimal health and ultimately to dysfunction, to biological stimuli, genetic manipulation and therapeutical interventions. This kind of studies pointed hints to discover lipid biomarkers in relation to health promotion and disease prevention and facilitated the understanding of the complex regulatory mechanisms in humans or animals. Particularly, the introduction of the systems biology view will not only provide in-depth insights into the multi-target synergetic effects (which have hardly been used in modern drug discovery) but also can bridge Chinese Medicine (multi-target therapy) and Western Medicine (molecular pharmacology).