

Cover Page



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Chapter 7

Conclusions and perspectives

Conclusions

Type 2 diabetes mellitus (T2DM) leads to a number of serious medical complications (e.g. retinopathy, neuropathy, myocardial infarction), which are a major cause of morbidity, hospitalization and mortality in diabetic patients; and resulting in a major threat and financial burden for public health internationally [1-3]. Due to its multi-factorial causes resulting from the interaction between a genetic predisposition together with behavioral and environmental risk factors, the diagnosis and treatment of T2DM remains unsatisfactory. A personalized and system based strategy applied in the pre-diabetic stage is vital for diabetic prevention and management.

Emerging technologies have made it feasible to acquire high-throughput profiles of a whole organism's metabolic status (metabolite profiling or metabolomics) [4]. These techniques, which allow assessment of large numbers of metabolites that are substrates and products in metabolic pathways, are particularly relevant for studying metabolic diseases such as T2DM [4]. Metabolomics has been increasingly applied to study complex disease mechanisms to discover health-disease associated mechanistic biomarkers and is regarded as a unique bridge between different healthcare perspectives on personalized medicine [5, 6]. The concept of personalized health with system diagnostic principles has long been the basis of CM, in which the focus is on the overall maladjustments of functional status called 'syndrome type'[7]. The essence of 'syndrome type' is the human system imbalance resulting in the perturbation of the metabolic network [8]. If we can correlate the CM diagnosed syndrome types to the metabolomics quantified patterns, it may help us stratify the patients and push personalized medicine forward.

In light of this idea, the aim of this thesis was to combine metabolomics and CM diagnostic strategy to search for the diagnostic makers to subtype T2DM. The multi-component preparations or drugs to treat cardio-metabolic disorders were studied and assessed by metabolomics to understand the potential underlying multi-pathway effects, leading to a better understanding of the personalized health of T2DM.

In **Chapter 2**, an explorative study of 50 pre-diabetic males was designed, combining urine metabolomics with CM diagnosis to identify metabolic patterns. Due to the fact that CM diagnosis is conceptual and relies entirely on clinical signs discerned by CM physicians, the inter-physician consistency on CM diagnosis is of importance to be investigated at the first place. Three CM physicians reached 85% diagnosis consistency when the diagnostic principles were standardized and symptom descriptions were clarified in advance. Based on 3 different CM syndrome groups, two pre-diabetic subtypes can be identified by urine

metabolomics and had different urinary metabolic patterns; and one of which indicated more disturbances of carbohydrate metabolism and renal function. The identified urinary metabolites may be of special clinical relevance for easy screening for subtypes of pre-/diabetes, which may lead to a better understanding and improvement of personalized interventions for diabetics. This study set an example to combine non-invasive gas chromatography–mass spectrometry (GC-MS) urine metabolomics with CM personalized diagnosis to find metabolic subtypes in pre-diabetics; and proved that the improvement of diagnosis plays a key role in bridging between CM and western medicine on personalized healthcare.

Obesity is one of the major risk factors for T2DM. Compliance with lifestyle modifications such as reduced caloric intake and increased physical activity has proved to be difficult for the general population, meaning that pharmacological intervention may be the only recourse for some people with obesity [9]. Therefore, in **Chapter 3 and 4**, the effects of rimonabant and a multi-component preparation (SUB885C), both with effects of regulating weight and the improvement on cardio-metabolic risk factors, were assessed by lipidomics on mildly over-weight ApoE*3Leiden.CETP Mice. Rimonabant is a selective cannabinoid-1 receptor antagonist for treatment of obesity and targets the endocannabinoid system. SUB885C is developed according to the principles of CM containing eight natural ingredients. The core formula is used in China for treatment of metabolic syndrome and early stage T2DM with obesity. For rimonabant and SUB885C, little is known about the impact on the regulation of lipid metabolism in the early stage of obesity. A 4-week rimonabant intervention brought a significant reduction of the body weight, yet a moderate effects on lipid profile and limited lipid metabolism changes in the ApoE*3Leiden.CETP mice. Although no weight reduction was observed, SUB885C was able to produce multiple anti-atherogenic changes in lipids of the mice to improve metabolic parameters and lipid patterns, manifesting as decreased plasma cholesterol, (V)LDL, and triglycerides, increased HDL-C and a change in wide range of neutral lipids. These effects were comparable to those obtained with compounds belonging to known drugs (e.g. atorvastatin, niacin). *In vitro*, SUB885C extract caused adipolysis stimulation and adipogenesis inhibition in 3T3-L1 cells. These two studies successfully illustrated the power of lipidomics in unraveling intervention effects and can help researchers pinpoint novel ways to treat lifestyle-related metabolic abnormality.

Panax ginseng C. A. Mey (often simply referred to as ginseng) is reported to have effects to treat diabetic symptoms and is widely used in China as the dietary or drug ingredient for daily health maintenance. So far no research has been performed to determine how the ginseng's growth year and its related quality

Conclusions and perspectives

affect its therapeutic efficacy. In **Chapter 5**, lipidomics was applied to evaluate the effects of the root of ginseng grown for 3–6 years on the regulation of hyperglycemia and dyslipidemia in a diabetic Goto-Kakizaki (GK) rat model. By applying liquid chromatography–mass spectrometry (LC-MS) based lipidomics, measuring biochemical parameters, and profiling the components of ginseng roots of different ages, we demonstrate that ginseng roots show growth age-dependent therapeutic effects on hyperlipidemia and hyperglycemia in diabetic GK rats. These effects may be linked with the age-dependent variations in concentrations of specific bioactive ginsenosides in the ginseng roots. The ≥ 4 year ginseng roots contain bioactive ginsenosides that may prove to be valuable in the development of drugs or dietary supplements to regulate lipid levels and increase glucose tolerance. This study introduced novel systems biology-based approaches for linking biological activities with potential active components in herbal mixtures.

Chapter 6 reports the preliminary development of a fast plasma lipid analysis platform by direct nanospray infusion using a TriVersa chip-based instrument in combination with high resolution LTQ-Orbitrap mass spectrometer. The modified Folch method was applied for the plasma lipid extraction. With the sample volume of 5 μ L plasma, a snapshot of the following lipid classes can be obtained within 5 minutes per sample: triacylglycerols, cholesterol esters, phosphatidylcholines, lyso-phosphatidylcholines, sphingomyelins and fatty acids. When compared with the validated LC-MS method, the fast method is equivalent to it for high abundance lipids; yet it is less precise than LC-MS method. However, it still holds promise for the fast lipids analysis for plasma samples and worth further development and validation.

To sum up, the early metabolomics studies carried out in this thesis set an example to combine analytical bioscience, clinical approach and other health system perspectives such as CM to provide the systems biology view on the metabolism patterns of T2DM and the intervention effects of possible drug and dietary approaches. It is promising to fusion metabolomics and CM diagnosis to search for subtypes of T2DM. Metabolomics, lipidomics in specific, is able to investigate multi-dimensional pharmacological effects and detect potential biomarkers related to these effects. These findings can be used to develop future new research strategies and products at the nutrition-pharma interface to manage T2DM and related metabolic risk factors. However, it will only be realized when analytical methods will further improve, and system biology based metabolomics approaches are more integrated in the medical research paradigm.

Perspectives

Improved diagnosis to stratify patients is important to push personalized medicine forward

The only one human study reported in this thesis (**Chapter 2**) showed the promise to fuse metabolomics and CM diagnosis to search for subtypes of T2DM. However, it is an early phase investigation on subtypes of pre-diabetes based on a small number of study subjects and CM physicians. As such whilst this data is very promising, there needs to be larger investigations using more subjects and CM physicians to demonstrate the further reliability and external validity/generalization ability of this novel approach to diagnosing pre-diabetic subtypes. Future studies are needed to validate the subtypes yielded in the current study and to assess the time dependent response of these subtypes to challenge test or metabolic drug intervention.

This study proved that the understanding and improvement of diagnosis plays a key role in bridging between CM and Western medicine (WM) on personalized healthcare. Stratifying patients on molecular biomarker profiles is a key step towards treatment responder/nonresponder differentiation and personalized medicine. The CM method of qualitative subtyping could be of use to help decide the course of treatment for patients in modern medicine, and provide momentum for the move towards personalized medicine [13]. A diagnostic tool such as a questionnaire can be designed, combining CM symptoms and WM quality of life items, to search for patients subtypes based on different health and disease related manifestations.

Interventions for T2DM

It is obvious that multiple mechanisms are involved in T2DM development, and so far no universal cure exists for all of the metabolic abnormalities that are embodied in this disease. For example, drugs aimed at metabolic targets, such as the enhancement of fatty acid oxidation, may have desirable effects on hepatic insulin action and steatosis, but possible deleterious effects in muscle or islet β -cells [9]. Moreover, drugs that stimulate insulin secretion beyond the already elevated levels of the obese and insulin-resistant state may eventually cause β -cell stress and permanent damage [9]. It is unlikely that effective treatments will be based on single bioactive compounds. These complexities may force a focus on combined drug/nutrition therapies with individualized optimization.

To restore energy balance and improve metabolic risks, rimonabant, a cannabinoid-1 receptor antagonist which regulates central appetite control in

Conclusions and perspectives

response to endogenous ligands, used to show promise. However, it has incompletely characterized peripheral effects, and has been associated with psychic (neurologic, mental) side-effects such as depression and even suicide attempt. Moreover, the lipidomics response to an intervention of rimonabant in transgenic mice, as reported in **Chapter 3**, only showed moderate effects in early stage obesity. Projects are underway to develop cannabinoid-1 receptor blockers that do not pass the blood-brain barrier (to prevent mental side effects) are non-brain penetrant and act only on peripheral cannabinoid-1 receptor [10]. Metabolomics can again be a good method to investigate the effects and mechanism of such agents.

Meanwhile, pharmacological strategies for primary prevention are increasingly focusing on the use of low-dose drug combinations. An example is the development of “polypill” concepts with a statin, one or more anti-hypertensive compounds and acetylsalicylic acid to reduce risks for cardiovascular disease in middle-aged individuals [11]. While at the same time it has been shown that dietary measures such as “polymeal” may be of comparable efficacy or even provide a “more natural, safer and probably tastier alternative” than a polypill [12]. Therefore, T2DM prevention through nutritional intervention is realized to be crucial for increased human quality of life. Metabolomics based nutritional profile is useful to give indications for personalized intervention that can influence on multiple targets in the human system with fewer side-effects. For example, an intervention with selected dietary products affected inflammatory processes, oxidative stress, and metabolism in humans, as shown by large-scale profiling of genes, proteins, and metabolites in plasma, urine, and adipose tissue [13]. To make it possible for health-care and nutrition practitioners to give nutrition recommendations, more trials using metabolomics and a suitable database based on a large number of measurements of accurate metabolite concentrations from healthy people might be necessary [14]. Furthermore the concept of health promotion alongside disease management will help to improve the current healthcare system [11]

Such new insights and leads for dietary prevention or intervention can also be acquired from CM where the gap between food and drugs is small. The study results of the multi-components preparation SUB885C and ginseng reported in **Chapter 4 and 5** [15, 16] may shed light on this approach. Both SUB885C and ginseng showed beneficial effects on dyslipidemia and may have influence on multiple pathways, which need further investigation. In China a remarkable high number of preparations have been handed down over the centuries with documented activity related to clinical features of what is now described as metabolic syndrome. The diabetic regulation health claims of these Chinese herbs included increasing insulin, decreasing blood glucose, increasing glucose

metabolism, stimulating pancreatic function, and alleviate diabetes-induced oxidative stress by inhibiting lipid peroxidation [17-19]. Only a few side effects of Chinese herbs used in treating diabetes have been reported, however, further comprehensive studies are warranted as evidence is still scarce with likely underreporting [19].

The use of Chinese herbal medicines, either as food ingredients or drug therapy, in diabetes seems promising but still far from proven. First of all, the lack of standardization in quality control and quality assurance in producing the herb and herbal products often leads to inconclusive intervention results. **Chapter 5** clearly illustrated the age-dependent effects of ginseng intervention and these age-dependent effects may be due to the fact that the ratios and concentrations of specific ginsenosides in the ginseng roots change during growth [15]. The possibilities to evaluate the quality control and analyze the subtle and multiple-pathway effects of Chinese herb or/and preparations have increased by the developments in systems biology-based metabolomics and specific animal models, as shown in **Chapter 4 and 5**.

In addition, many of the herbs are still untested or tested only in limited trials, thus further clinical trials are needed to evaluate their effects and mechanisms [19]. To avoid the underestimation of the Chinese herb intervention, it is suggested that study subjects in such clinical trials should at first be stratified according to CM diagnosed subtypes as the CM diagnosis is on the syndrome types instead of a disease in WM perspective. Therefore, improved diagnosis and intervention go hand in hand, and metabolomics can certainly contribute to find and understand the molecular phenotypes.

The challenge of metabolomics study and the future direction

With respect to the technology of metabolite profiling, further progress is required to determine the chemical identity of peaks that can be determined with metabolite-profiling methods. Given the fact that the phenotype of any biological system is largely determined by its metabolite composition, the future development of metabolite-profiling technologies is of crucial importance to biomedical research [20].

It seems wise to combine the untargeted metabolomics platform with targeted platforms in the same study. As for an untargeted global metabolite analysis, so many of the changes detected are possibly associated with high concentration metabolites. Many of the metabolites detected in studies by GC-MS are involved in amino acid metabolism, the Krebs cycle, glycolysis and β -oxidation, and thus it is perhaps not so surprising that the approach detects changes in these pathways [21]. To additionally apply a targeted approach where a number of predefined

Conclusions and perspectives

metabolites are targeted, this has the advantage that this type of approach can also be readily made quantitative by nature and more biological interpretation of results can be drawn.

Lipidomics measures all or subsets of lipids and provides a thorough perspective to study intervention induced lipid changes and metabolism in the complex biological system. To study more in-depth the involvement of specific lipid species and pathways, more understanding of lipid metabolism is needed. T2DM is not only a disorder of glucose metabolism, but also associated with a cluster of interrelated plasma lipid and lipoprotein abnormalities. Combining lipidomics with biochemically measured lipid profiles (e.g. total cholesterol, triglycerides, HDL-CI, etc) can provide us with a better understanding of lipid metabolism. **Chapter 3-5** describes the successful application of this approach, using LC-MS based lipidomics, to investigate the changes in the lipidome of animal models following interventions and illustrated possible underlying mechanism how lipid were re-distributed in the biological system after intervention.

The amount of information generated with metabolomics studies does not in itself a guarantee for the discovery of a biomarker or a metabolic pattern. It is important to carry out biomarker validation and follow-up studies to complete the cycle of hypothesis generation and hypothesis testing. Approaches can be like MolPAGE project [22], using large cohorts and extensive statistical material from clinical settings will enable the method to expand into a better understanding of the disease pathophysiology, which could lead to the biomarkers for clinical settings.

Another obstacle to progress in biomarker research resides as much in the culture and organization of academic research as in deficiencies in collaborative approaches. To become clinically useful, biomarker research must operate more like the large, collaborative networks mobilized for international genome-wide association studies and the multi-institution, multi-investigator big-science projects, involving industry and experts in molecular biology, genetics, analytical chemistry, computation, engineering, clinical-trial design, epidemiology, statistics, regulation and health-care economics [23]. To reach this, organizational and funding reforms should persuade the research community to adopt common standards and a cross disciplinary, systems-based approach to biomarker discovery and validation [23].

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Conclusions and perspectives

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