Metabolomics: Towards Understanding Traditional Chinese Medicine

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Key words
- metabolomics
- biomarkers
- traditional Chinese medicine
- systems biology
- NMR
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- LC-MS
- metabolic diseases

Abstract

Metabolomics represent a global understanding of metabolite complement of integrated living systems and dynamic responses to the changes of both endogenous and exogenous factors and has many potential applications and advantages for the research of complex systems. As a systemic approach, metabolomics adopts a "top-down" strategy to reflect the function of organisms from the end products of the metabolic network and to understand metabolic changes of a complete system caused by interventions in a holistic context. This property agrees with the holistic thinking of Traditional Chinese Medicine (TCM), a complex medical science, suggesting that metabolomics has the potential to impact our understanding of the theory behind the evidence-based Chinese medicine. Consequently, the development of robust metabolomic platforms will greatly facilitate, for example, the understanding of the action mechanisms of TCM formulae and the analysis of Chinese herbal (CHM) and mineral medicine, acupuncture, and Chinese medicine syndromes. This review summarizes some of the applications of metabolomics in special TCM issues with an emphasis on metabolic biomarker discovery.

Abbreviation

LC: liquid chromatography

Introduction

In the last two decades, metabolomics are considered important tools to be applied and utilized to understand the biology of an organism and its response to environmental stimuli or genetic perturbation. Metabolomics (a flowchart is shown in **Fig. 1**) was originally proposed as a method of functional genomics [1], but its utility extends well beyond that – it is useful whenever an assessment of changes in metabolite levels is needed. Metabolomics (or metabonomics) is used for assessing responses to environmental stress [2,3], comparing mutants [4] and different growth stages [18,19], for drug [5] and natural products [22] discovery, toxicology [6,7], nutrition [8–12], genetic manipulation [13], cancer [14–17], and diabetes [20,21]. Indeed, metabolomics is the study of global metabolite profiles in a system (cell, tissue, or organism) under a given set of conditions and has its roots in early metabolic profiling studies, but is now a rapidly expanding field of scientific research, which has justifiably taken its place alongside genomics, transcriptomics, and proteomics as one of the latest and most exciting "-omic" sciences [23]. The global metabolite profiling involves measuring low molecular-weight metabolites (< 1 kDa) in complex biofluids/tissues to study perturbations in response to physiological challenges, toxic insults, or disease processes [24]. Metabolites are the end products of cellular regulatory processes and can be regarded as the ultimate response of biological systems to control genetic or environmental changes [25]. A deeper understanding of global perturbations in biochemical pathways in complex diseases could provide valuable insights about the mechanisms of disease. In addition, metabolomics has the potential to enable mapping of early biochemical changes in disease and hence provide an opportunity to develop predictive biomarkers that can trigger earlier interventions.

TCM, a complex medical science, reflects traditional Chinese culture and philosophical principles, embodies rich dialectical thought, places the human body into a large system for observation and adjusts humans to remain in a healthy status. Entering the 21st century, TCM is getting

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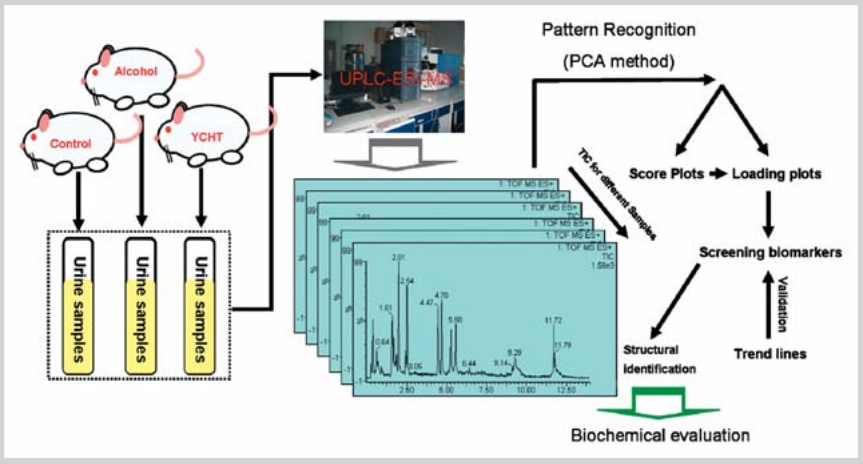

Fig. 1 Flowchart of metabolomic analysis.

more and more popular in the whole world for improving health conditions of human beings and preventing or healing diseases, and is especially showing great advantages in early and combination intervention, personalized therapy, etc. The international community has attached increasing importance to TCM [26]. However, like almost all other ethnopharmacologies, TCM faces severe challenges and suffers from insufficient scientific research owing to the lack of modern and technologic approaches; this restricts the development of TCM in the world. Chinese medicine is not yet an integral part of the standard healthcare system in Western countries due to a lack of scientific evidence for its efficacy and safety as well as a language and cultural barrier [27]. Fortunately, metabolomics adopts a "top-down" strategy to reflect the function and metabolic changes of complete organisms caused by interventions in a holistic context. This property coincides with the holistic thinking of TCM and has the potential to impact our understanding of Chinese medicine theory. Recently, a wide range of metabolomic analytical techniques are widely used in the modern research of TCM. Metabolomic analysis will facilitate the modernized study of TCM syndromes, provide an in-depth understanding of the TCM theory, help predict the disease on-set and achieve a comprehensive evaluation of clinical efficacy, safety, and action mechanisms of TCM formulae. Metabolomics combined with TCM methodologies will provide a new pathway and methodology for the study of complicated systems theory of TCM and its modernization. Especially the advances in the high-throughput and comprehensive research technologies and the idea of metabolomics provides new strategies for the analysis of active components in the formulae of TCM *in vivo*. Furthermore, the initiatives of metabolomics may pave a new way to explain the action mode of TCM in light of modern sciences and so contribute to establish a new technique platform for evaluating the efficacy of the TCM formulae. It is believed that with the further development of metabolomic analytical techniques, especially multi-analysis techniques, metabolomics will greatly promote TCM research and be beneficial to its modernization as well as establish international standards for it. Thus, we draw attention to applicable approaches of using metabolomic techniques to resolve special TCM issues and focuse particularly on the potential of metabolomics to contribute to biomarker discovery in TCM research.

Advantages of Metabolomics

The study of biological systems in a holistic manner (systems biology) is increasingly being viewed as a necessity to provide qualitative and quantitative descriptions of the emergent properties of the complete system. Systems biology performs studies focused on the complex interactions of system components, emphasizing the whole system rather than the individual parts. Metabolomics is one functional level tool being employed to investigate the complex interactions of metabolites with other metabolites but also the regulatory role metabolites provide through interaction with genes, transcripts, and proteins (e.g., allosteric regulation). Technological developments are the driving force behind advances in scientific knowledge. Recent advances in the two analytical platforms of mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy have driven forward the discipline of metabolomics [28–32]. Technological advances in NMR and mass spectrometry have opened a new chapter in biochemistry by using metabolomics as an approach to study metabolism and its regulation in relation to genetic, disease, and environmental factors. NMR is one of the most commonly used technologies in metabolomics research, providing detailed information on the molecular structure and probing metabolite molecular dynamics and mobility [33]. The high selectivity of mass spectrometry with low-detection limits makes mass spectrometry an ideal tool for metabolomic applications. It requires separation of the metabolic components using either gas chromatography after chemical derivatization or ultra-performance liquid chromatography (UPLC). UPLC/MS is often used to obtain the largest possible biochemical profile information subset. It is a sensitive tool that can be used to characterize, identify and quantify a large number of compounds in a biological sample where metabolite concentrations might cover a broad range of information with regard to disease pathophysiology [34–36]. All metabolomic studies result in complex multivariate datasets that require visualization software as well as chemometric and bioinformatic methods for interpretation. The application of software tools for the analysis of the information contained in a database can identify the signature of a disease and predict its risk and progression.

One of the major benefits of metabolomics in the study of disease and drug therapy is that metabolic profiling can usually be achieved using urine or plasma samples. The accessibility of urine and plasma clearly makes these samples ideal for large-scale re-

search [37]. Metabolomics data can be analyzed with a range of statistical and machine-learning algorithms. These algorithms can be classified within two major classes: unsupervised and supervised [38]. They can be useful in the identification of biomarkers [39,40]. Examples of unsupervised methods that have been routinely used in analyzing molecular fingerprinting data include principal component analysis (PCA) and self-organizing maps [41].

Metabolomics has become useful in many medicine areas as an aid to disease diagnosis or staging and as a tool to predict or monitor treatment response or toxicity. Considerable interest in the field of metabolomics is that of personalized health care, whereby an individual's drug treatment is tailored so as to achieve maximal efficacy while avoiding adverse drug reactions. Another more recent approach has been to use metabolomics to predict the metabolism of a dosed substance based on a pre-dose metabolic profile [42]. Metabolomics holds a comprehensive and non-invasive analysis of metabolic biomarkers that could detect early-stage disease, identify residual disease post-surgery and help to monitor treatment response. Additionally, metabolomics can be seen as bridging the gap between genotype and phenotype, providing a more comprehensive view of how cells function, as well as identifying novel or striking changes in specific metabolites [25,43]. In the future, metabolomics may enable us to develop new approaches for improving the health conditions of human beings and preventing or healing diseases, and is especially showing great advantages in early and combination intervention, personalized therapy, etc. [44,45].

Challenges for TCM

TCM has been practiced for thousands of years and the written documents showed sophisticated theories for TCM even in its early forms although these theories were established on a philosophical basis and clinical experiences rather than science perhaps owing to the fact that this medical system had been developed well before modern science was born. Like almost all other traditional medicines, the practice of TCM suffers, at present, from insufficient modern scientific research. For example, many treatments of TCM are still practiced in their original form, and although they have been effective in the therapy of many conditions, especially chronic ones, they lack the necessary definition of a molecular mechanism and sometimes even of a molecular basis. Prescribed medicines in TCM are normally mixtures of a number of plants, and their composition is not well defined. There have been many recent attempts to address these issues but most of them were still based on the "reductionism" philosophy. Therefore, there is a clear urgency of scientific research in terms of quality control, clinical efficacy, and molecular mechanisms of traditional medicines, which undoubtedly demands a significant amount of analytical power and effort.

Metabolomic Applications of TCM

Metabolomic evaluation of formulae

Wang et al. evaluated metabolomic characters of the hepatotoxicity induced by alcohol and the intervention effects of Yin Chen Hao Tang (YCHT), a classic traditional Chinese medicine formula composed of Flos Artemisiae, Gardeniae Jasminoidis, and Fructus and Radix et Rhizoma Rhei for the treatment of jaundice and liver disorders in China [46]. The greatest difference in metabolic profiling was observed in alcohol-treated rats compared with control and YCHT-treated rats (**Fig. 2**). The number of positive ions, *m/z* 664.3126 (9.00 min), was elevated in the urine of alcohol-treated rats, whereas ions with *m/z* 155.3547 (10.96 min) and 708.2932 (9.01 min) were at a lower concentration compared with those in the urine of control rats. However, the number of these ions was not statistically different between control and YCHT-treated rats (**Fig. 3**). The ion with m/z 664.3126 was found to correspond to ceramide (d18:1/25:0), providing further support for an involvement of the sphingomyelin signaling pathway in alcohol hepatotoxicity and the intervention effects of YCHT (**Fig. 4**). More recently, Wang et al. explored the excretion pattern of low molecular mass metabolites in the male Wistar-derived rat model of kidney yin deficiency induced with thyroxine and reserpine as well as the therapeutic effect of Liu Wei Di Huang Wan (LW), one of the most important Chinese formulas consisting of six herbs including *Rehmannia glutinosa* Libosch. (family: Scrophulariaceae), *Cornus officinalis* Sieb. (family: Cornaceae), *Dioscorea opposite* Thunb. (family: Dioscoreaceae), *Alisma orientale* (G. Samuelsson) Juz (family: Alismataceae), *Poria cocos* (Schw.) Wolf (family: Polyporaceae), and *Paeonia suffruticosa* Andrews (family: Paeoniaceae), and widely used in eastern Asia for treating kidney yin deficiency in China [47]. The results showed that the changes in metabolic profiling were restored to their baseline values after treatment with LW according to the PCA

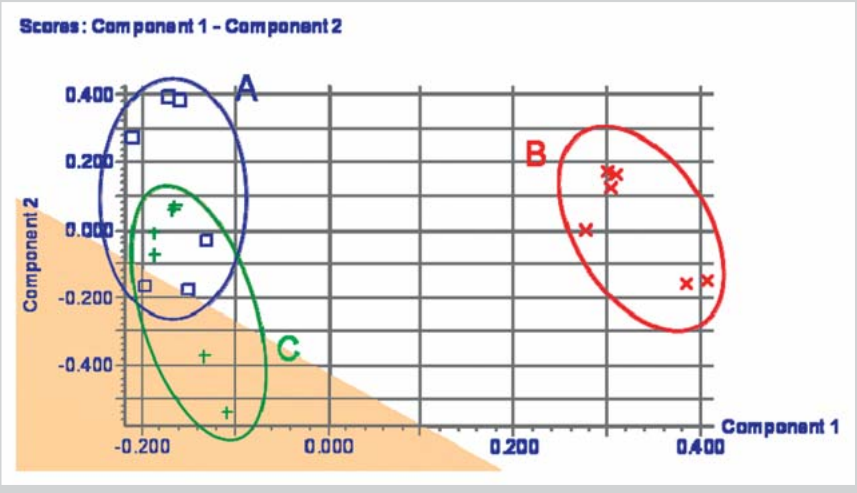

Fig. 2 Resulting score plots from PCA of reversed-phase UPLC-ESI-TOF-MS data obtained from (**A**) control rats, (**B**) alcohol-treated rats, (**C**) YCHT-treated rats urine samples collected from day 7. Symbols: control rat (□), alcohol-treated rat (×), and YCHT-treated rat (+).

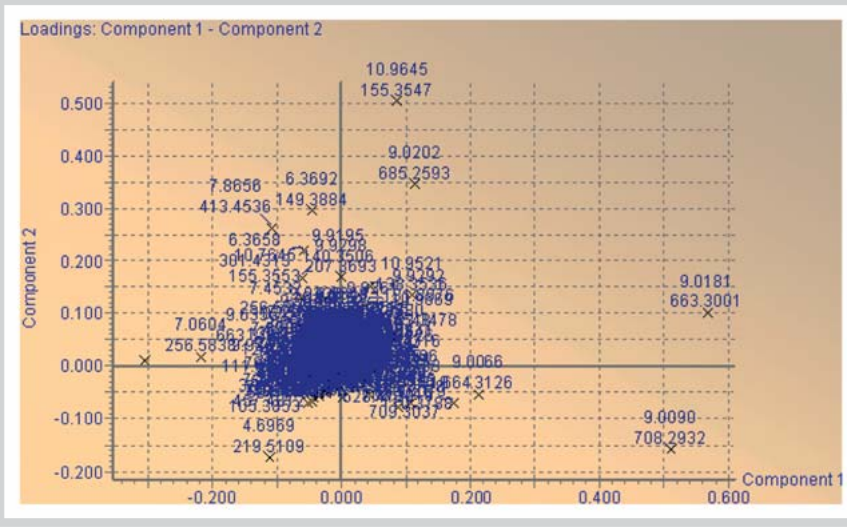

Fig. 3 Resulting loadings plot from PCA of reversed-phase UPLC-ESI-TOF-MS data obtained from control rats, alcohol-treated rats, and YCHT-treated rats urine samples collected from day 7.

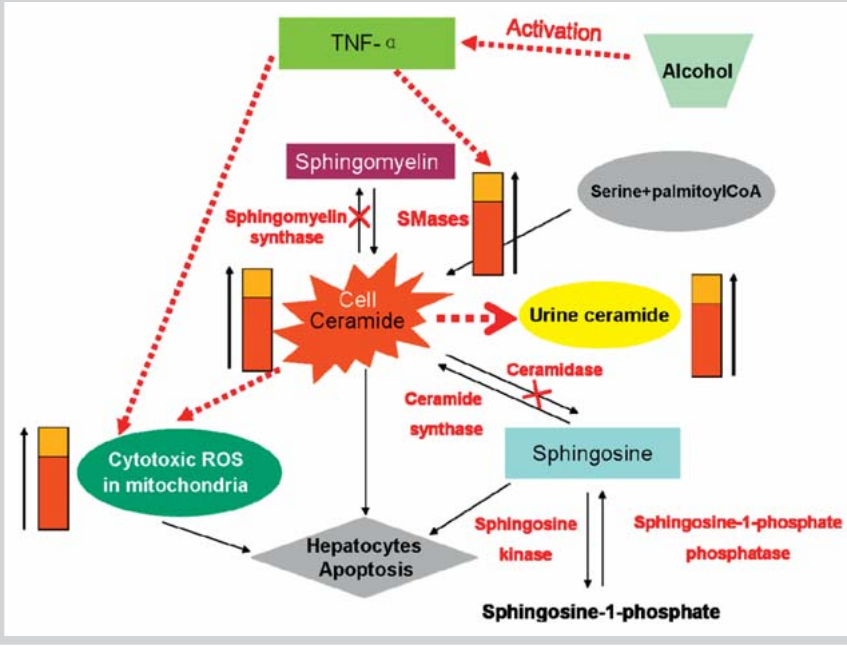

Fig. 4 The proposed metabolic pathway for explanation of the relationship between alcohol hepatotoxicity and the increased content of ceramide (d18:1/25:0) in urine.

score plots (**Fig. 5**), indicating 20 ions (8 in the positive mode, 14 in the negative mode, and 2 in both) as "differentiating metabolites" (**Fig. 6**). PCA score plots separated urine samples into different blocks, and samples subjected to the same treatment were located on the same trajectory, indicating that treatments have greatly disturbed the normal urine metabolic profiles of rats. There were different phenotypes of metabolites based on HPLC-UV urinary profiling after administration of LW pills or carrageenan-stimulated inflammation model, and those could be conveniently discriminated by PCA. In addition, the results also showed that LW Pills could restore the metabolite network that was disturbed by inflammation, which would be a proof of the therapeutic efficacy of LW pills on inflammation from a metabolomics study [48]. A urinary metabolomics method based on the UPLC-MS was used to evaluate the efficacy and study the action mechanism of Xindi soft capsules, consisting of sea buckthorn flavanoids (e.g., quercetin, kaempferol, and isorhamnetin), which is a TCM preparation against blood stasis [49]. Compound Danshen tablets, a herbal (*Salvia miltiorrhiza* Bge.) compound preparation, presented protective effects on myocardial ischemia by reversing potential biomarkers to sham levels, especially for the four metabolites in the pathway of purine metabolism (hypoxanthine, xanthine, inosine, and allantoin) [50]. Damage of mitochondria, disorder of energy metabolism, and osmoregulation were observed in a cyclophosphamide-caused blood deficiency model by an NMR-based metabolomics method. Siwutang, a novel prescription consisting of *Paeonia lactiflora* Pall, *Angelica sinensis, Rehmannia glutinosa, and* Rhizoma Chuanxiong could improve these effects [51].

Metabolomic analysis of CHM

CHM has long been used for disease prevention and therapy in China and is becoming increasingly important in the West [52]. Herbal medicine has been the source of many drugs used in modern therapeutics, and particularly in the case of anticancer drugs, more than 50% originally came from natural products. However,

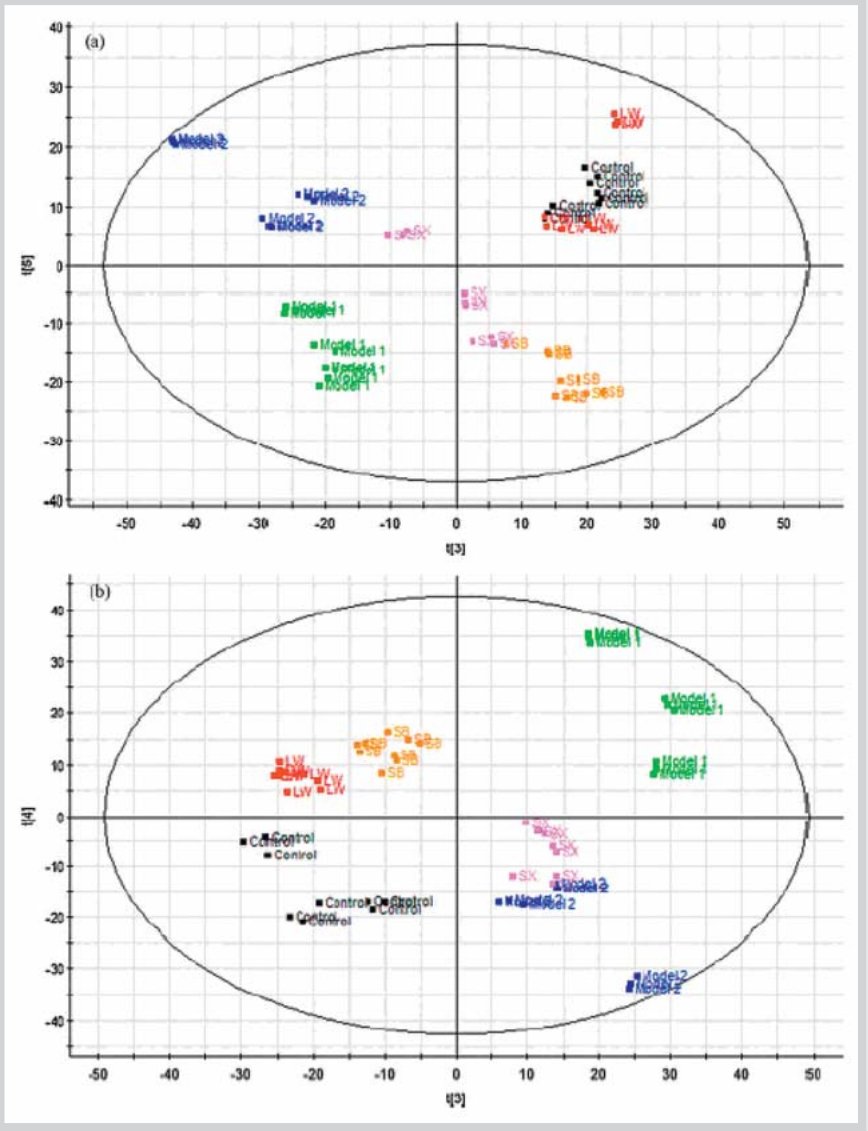

Fig. 5 PCA score plots of urine samples collected from different treatment groups of rats (**a**) in negative ESI mode and (**b**) in positive ESI mode. Key: ■ Control; ■ LW; ■ Model 1; ■ Model 2; ■ Sanbu; ■ Sanxie.

due to the painstaking way of conventional lead-finding, the attention towards CHM has been deviated in the last decades. A new strategy for the detection of active compounds is necessary to get natural product research out of its stalemate. Metabolomics, with its holistic approach and the possibility it provides for the simultaneous detection of all sorts of metabolites, has the potential to be instrumental for this new approach. A combined GC/MS and LC/MS metabolic profiling strategy indicates that *Tripterygium wilfordii* Hook. F caused a time-dependent toxic effect at a high dose as revealed by the perturbed metabolic regulatory network involving disorders in energy metabolism, elevated amino acid, and choline metabolism pathways, as well as altered structure of gut flora [53]. Urinary metabolic perturbations associated with liver toxicity induced by Huang-yao-zi (root of *Dioscorea bulifera* L.) were studied using NMR to determine the correlations between metabolomic profiling and histopathologic/biochemical observations and to discover biomarkers for liver toxicity, indicating metabolic changes observed in urine samples in response to Huang-yao-zi treatment. In addition, the mechanism associated with oxidative injury of hepatic mitochondria was investigated [54]. HPLC-MS/MS-based metabolomics method was used to find the possible biomarker of Rhizoma Coptidis in rat urine. 169 kinds of biomarkers were found. The result was consistent with the pharmacological effects of R. Coptidis, such as anti-inflammatory, anticentral nervous system, and energy metabolism inhibition [55]. Berberine might play a pivotal role in the treatment of type 2 diabetes through downregulating the high level of free fatty acids. Comprehensive metabolomic measurements are potentially very useful for studying the mechanisms of action of traditional Chinese medicines [56]. Combined NMR and LC-DAD-MS analyses reveal comprehensive metabolomic variations for three phenotypic cultivars of *Salvia miltiorrhiza* Bunge [57]. NMR-based metabolomics is an attractive method for nonselective and comprehensive analysis of *Ginkgo* extracts, which are very complex mixtures prepared from raw leaf extracts by a series of extraction and prepurification steps [58]. *Ginkgo biloba* leaves exert multidirectional lipid-lowering effects on the rat metabonome, including limitation of the absorption of cholesterol, inactivation of HMGCoA, and favorable regulation of profiles of essential polyunsaturated fatty acid [59]. Recently, changes of metabolites in rat urine after treatment with *Aristolochia fangchi* decoction were studied by the metabolomic method [60]. High-dose *Aristolochia fangchi* can induce nephrotoxicity, and its seriousness corresponds to the duration of ad-

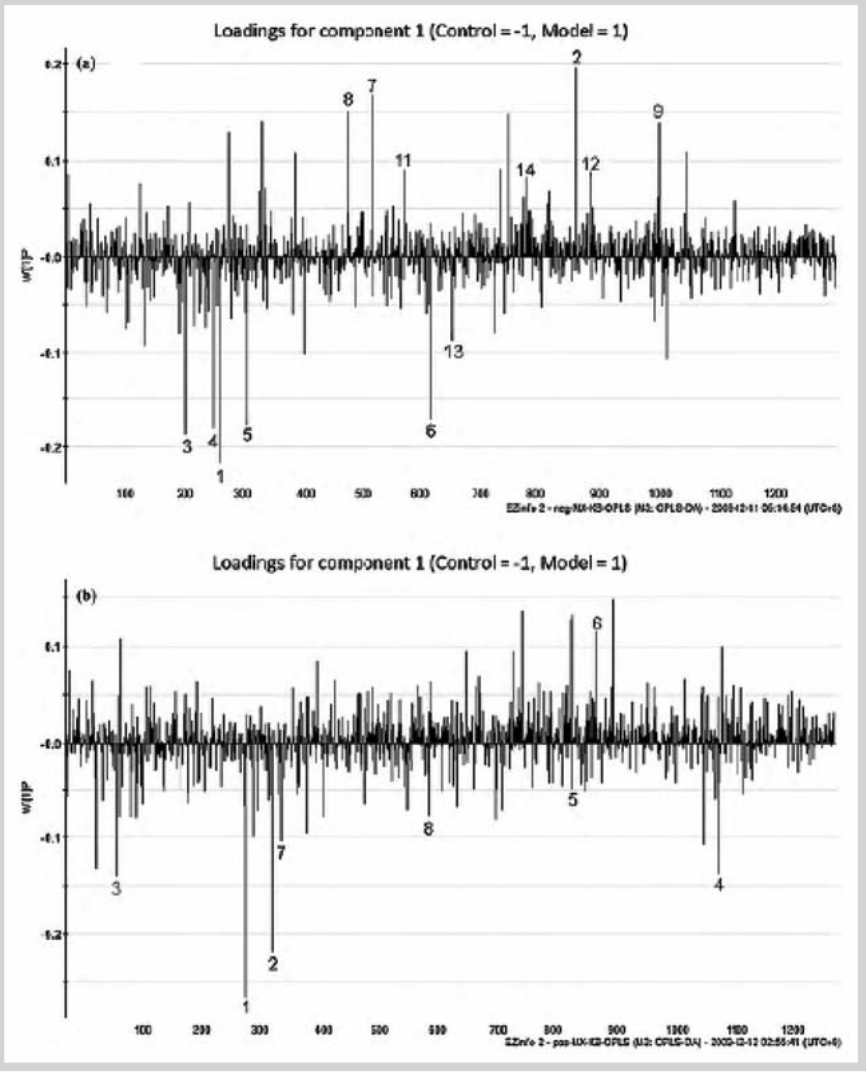

Fig. 6 Component loadings in (**a**) negative ESI mode and (**b**) positive ESI mode.

ministration. *Aristolochia fangchi* may also have toxicity on the liver. The results suggested that this metabolomic approach is a promising methodology for the rapid *in vivo* screening of nephrotoxicity associated with ingesting multi-ingredient medicinal herb supplements [61]. Aristolochic acids, naturally present in *Aristolochia* plant species that have been used in CHM containing a mixture of varying herb species, were identified by UPLC-MS-based methodology [62]. A LC/MS metabolomics approach was applied to characterize the aging of rats and the antiaging effect of total flavones of *Epimedium*, a traditional Chinese medicine, indicating that aging could be characterized by changes of lipid metabolism and accumulation of free radicals. The antiaging effects of total flavones of *Epimedium* might be due to the intervention on lipid metabolism and its property of antioxidation [63]. Phenotype of aging at different levels demonstrates a common age-dependent trend. *Epimedium* flavanoids can reverse this age-dependent change at different levels in a synchronous manner [64]. The total flavones of *Epimedium* administration can markedly influence the aging process and exert antiaging effects, which might due to the melioration of pyruyate metabolism and oxidative phosphorylation [65]. Significant differences in endogenous metabolite profiles were observed in the intervention rats, and the abnormality of metabolism recovered towards the normal level after administration with *Epimedium brevicornum* extract. Four active constituents of *Epimedium brevicornum* Maxim were found in the blood circulation of kidney-deficient rats and two of the metabolites in the urine. It suggests that the metabolomic approach is a potentially powerful tool to explore the therapeutic basis and to clarify the possible action mechanism of TCM herbs [66].

Metabolic changes in Wistar rats caused by *Aconitum* alkaloids aconitine, mesaconitine, and hypaconitine, which are the main toxic components of the traditional herbal medicine Fu Zi (*Aconitum carmichaelii* Debx.) were investigated by means of integrated analysis of two metabolomic approaches. Metabolites with significant changes or with a tendency to change in the aconitine and mesaconitine groups were dissimilar, suggesting a possible difference in the acute toxicity mechanisms of these alkaloids [67]. A metabolomic investigation of intoxication with *Aconitum sp.* alkaloids was carried out: they can cause metabolic disorders in rats. The toxicity and corresponding mechanism of hypaconitine was different from those of aconitine and mesaconitine, based on the differences of perturbed metabolic patterns between groups [68]. The effect mechanism and potential biomarkers of the toxicity of Hei-Shun-Pian, the processed lateral root of *Aconitum carmichaelii* Debx. (Ranunculaceae), on the metabolic profile of rats, suggests a toxic effect of Hei-Shun-Pian on the rat heart in a dose-dependent manner [69]. *Artemisia afra*

has been used as an infusion to treat malaria throughout the southern parts of Africa, in much the same way as the antimalarial plant *Artemisia annua* in China. Liu et al. had used metabolomics to investigate the ethnopharmacological use of *Artemisia afra* with NMR spectroscopy and multivariate data analysis [70]. Their findings show that there is no *in vitro* activity, and a list of the identified metabolites causing the metabolic differences is presented. Artemisinin has been proven to be an effective antimalarial compound, especially for chloroquine-resistant and cerebral malaria. In order to get new clues about artemisinin biosynthesis, metabolic profiling by GC and GC-MS was applied to compare the secondary metabolites of two *Artemisia annua* L. genotypes. It could be shown that there were clear differences in terpenoids and artemisinin metabolism between different growth stages and genotypes [71].

Metabolite profiling of five medicinal *Panax* herbs, including *Panax ginseng* (Chinese ginseng), *Panax notoginseng* (Sanchi), *Panax japonicus* (Rhizoma Panacis Majoris), *Panax quinquefolium* L. (American ginseng), and *P. ginseng* (Korean ginseng), was performed using UPLC-MS and a multivariate statistical analysis technique. PCA of the analytical data showed that the five *Panax* herbs could be separated into five different groups of phytochemicals. The chemical markers such as ginsenoside Rf, 20(S)-pseudoginsenoside F11, malonyl ginsenoside Rb1, and ginsenoside Rb2, accountable for such variations, were identified through the loadings plot of PCA, tentatively by the accurate mass of TOF/MS, and partially by a reference standard [72]. Six different types of ginseng roots from China and Korea could be easily differentiated by NMR-based metabolomics [73]. Pharmacodynamic effects of the ginsenoside Rg3 on the urine metabonomes of healthy and liver-tumor-bearing rats have been investigated. Seventeen biomarker candidates including three apolar metabolites were detected for global analysis of highly complex biosamples [74]. This approach may not only increase the number of discovered biomarkers but consequently improve the comprehensive information on metabolic changes.

Liang et al. used LC/MS to analyze 16 saponins simultaneously, and the developed methodology could effectively break the application bottleneck on the quantitative analysis of multi-component LC/MS data and be applied widely in related fields for multi-component analysis, especially in CHM research [75]. Concurrently, a number of metabolites involved in glucose metabolism, citric acid cycle, and amino acid metabolism were affected immediately after the intake of green tea, and the proposed approach provided a more comprehensive picture of the metabolic changes after intake of green tea in human urine [76]. Interestingly, green and black tea intake had a different impact on endogenous metabolites in urine and plasma. Green tea intake caused a stronger increase in urinary excretion of several citric acid cycle intermediates, which suggests an effect of green tea flavanols on human oxidative energy metabolism and/or biosynthetic pathways [77]. The metabolic strategy has shown its potential in optimization of harvest time and chemical markers screening of tangerine peels, herbal materials of two coupled traditional Chinese medicines, Pericarpium Citri Reticulatae and Pericarpium Citri Reticulatae Viride, which will open a wide perspective in the analysis of "coupled TCMs" [78]. Major metabolite of both arecoline and arecaidine, N-methylnipecotic acid, is a novel metabolite arising from carbon-carbon double-bond reduction. Another unusual metabolite found was the monoacylglyceride of arecaidine. It was shown which role is played by these uncommon metabolites in the toxicology of arecoline and arecaidine [79]. Evocarpine, from the Chinese herb *Evodia rutaecarpa*, is not transported by p-gp and showed only slight toxicity at the highest test concentration of 30 µM [80]. *Evodiae rutaecarpa* has changed the endogenous metabolites of rats and can provide the base for the further research on the interpretation of drug property [81]. Molecular compositions of rosemary (*Rosmarinus officinalis* L.) extracts and their dependence on extraction solvents, seasons, and drying processes were systematically characterized using NMR spectroscopy and multivariate data analysis. Results showed that the rosemary metabonome was dominated by 33 metabolites including sugars, amino acids, organic acids, polyphenolic acids, and diterpenes, among which quinate, cis-4-glucosyloxycinnamic acid, and 3,4,5-trimethoxyphenylmethanol were found in rosemary for the first time [82]. It can be concluded that the metabolomics are a potentially powerful tool to explore the therapeutic basis of TCM herbs.

Metabolomics in the study of mineral medicine

An important traditional Chinese mineral medicine, *Cinnabar*, has been widely used as a Chinese patent medicine ingredient for sedative therapy, induced disturbance in energy metabolism, amino acid metabolism and gut microflora environment, as well as slight injury in the liver and kidney, which might indirectly result from cinnabar induced oxidative stress [83]. The time-dependent biochemical variations induced by realgar were achieved using pattern recognition methods. They illustrated the high reliability of the NMR-based metabolomic approach for the study of biochemical effects induced by mineral medicine [84].

Metabolomics in acupuncture

Chinese acupuncture, handed down from the ancient times, still survives in clinical practice nowadays. At present, acupuncture has attracted extensive attention in the domestic and overseas circles in the TCM field. Numerous experimental studies have demonstrated that acupuncture can correct various metabolic disorders such as hyperglycemia, overweight, hyperphagia, hyperlipidemia, inflammation, altered activity of the sympathetic nervous system, and insulin signal defect, all of which contribute to the development of insulin resistance [85]. In addition, acupuncture has the potential to improve insulin sensitivity. These studies have revealed the mechanisms responsible for the beneficial effects of acupuncture, though further investigations are warranted. Functional dyspepsia acupuncture-treated patients showed significantly changed levels of leucine/isoleucine, lactate, and glucose, and a slightly changed lipids level towards those of the healthy controls, demonstrating the therapeutic effects of acupuncture on the relief of functional dyspepsia symptoms; this is a NMR-based metabolomic approach in the study of biological effects of acupuncture [86].

Metabolomics in Chinese medicine syndromes

Metabolomics, the analysis of the metabolite profile in body fluids or tissues, is being applied to the analysis of a number of different diseases as well as being used in following responses to therapy. There is good reason to think that metabolomics will find particular utility in the investigation of disease, which may have tissue specific and systemic components. Metabolomic analysis can inform us about ocular or other body fluids and can therefore provide new information on pathways and processes involved in these responses.

Metabolomics has been used in several animal models of human disease. A recent study was conducted by Qiu et al. to investigate

the metabolic responses to Herba Cistanches intervention in a rat model of hydrocortisone-induced "kidney-deficiency syndrome". It revealed that Herba Cistanches caused a systemic recovery from hydrocortisone- induced metabolic perturbation in rats [87]. The TCM concepts of "Xinxueyuzuzheng (heart blood stasis obstruction pattern)" and "Qiyinliangxuzheng (qi and yin deficiency pattern)" for myocardial ischemia rat models were constructed by Yan et al. Endogenous metabolites in rat plasma were identified, such as valine, serine, threonine, ornithine, hydroxyproline, lysine, 2-hydroxybutanoic acid, 3-hydroxybutanoic acid, galactofuranose, and inositol. These compounds were indicated as the potential biomarkers, suggesting that the two above-mentioned patterns are involved in dysfunction, oxidative stress, energy metabolism, and amino acid metabolism [88]. Chen et al. described the metabolomic study of a biochemical modification *in vivo* induced by a high dose of hydrocortisone, which led to a unique pathologic condition similar to the "kidney deficiency syndrome", an early stage of obesity and diabetes in TCM [89]. In the study, 24-h urine was collected pre-dose and at days 1, 3, 7, and 10 post-dose after rats were injected with hydrocortisone at 1.5 mg/100 g. The acquired data were transferred into Matlab to be processed using PCA. Results indicated clear and consistent biochemical changes following hydrocortisone intervention. It suggested that metabolomic approach could be used as a potentially powerful tool to investigate the biochemical changes of certain physiopathologic conditions such as metabolic syndrome, as an early diagnostic means.

Luo et al. determined the changes of the plasma metabolic phenotype in rats with chronic restraint stress (rats with syndrome of liver qi stagnation and spleen deficiency) [90]. Metabolomic analysis of rat plasma revealed different metabolic spectra between stress and control groups, which were consistent with alterations of *in vivo* metabolisms in rats under stress stimuli. Compared with the control group, rats with repeated stress displayed significant changes in spectral peak shapes of acetate, lactate, tyrosine, and low-density lipoprotein. These altered metabolites can be used as biomarkers of syndrome of liver qi stagnation and spleen deficiency. Therefore, metabolomics is an approach with good development prospects for studies of TCM syndromes.

Conclusion and Future Perspective

In this review, we delineate and discuss metabolomic approaches to resolve special TCM issues. Metabolomics, a new but rapidly growing field, has the potential to impact our understanding of TCM theory and molecular mechanisms of disease. Facing the complicated life phenomemon, metabolomics will be a powerful means to the research of a complex system theory and modernization of TCM. So that application of metabolomics for TCM will facilitate the understanding of the intrinsic quality of TCM syndromes and the evaluation of the therapeutic effects of its formulae, Chinese herbal and mineral medicine, as well as acupuncture, and explore the therapeutic basis and the possible action mechanisms of TCM. Combined metabolomics and TCM methodologies will also provide a new pathway and methodology for TCM and its modernization. Overall, with the further development of metabolomic analytical techniques, especially multi-analysis techniques, we eagerly expect that metabolomics will greatly promote TCM research and establish international standards, being beneficial to its modernization.

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