

# Metabolomics: A New Perspective to Persian Medicine

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## Abstract

**Background:** In recent years, research on how traditional medicine works and the relationship between medicine and classical medicine has increased. In this regard, the use of metabolomics is one of the main candidates that can reflect the state of the body's basic metabolism and may be able to explain a new way to explain the function of traditional medicine by evaluating its effect based on evidence and modernizing it under the supervision of new sciences.

**Materials and methods:** In this review study, in an advanced search from papers published in English language from 2003 to 2020 with key words of "traditional medicine", "temperament" and "metabolomics" in the databases of PubMed, Medline, Science Direct, Cochrane, Embase is used.

**Result:** Animal and human studies conducted to relate the concepts of Traditional Chinese Medicine and metabolomics. The results of studies showed the specific metabolic profile in subtypes of diseases based on theory of Chinese Medicine. In field of Persian Medicine (PM), limited researches have been published on the differentiation of proteomics and metabolites of individuals with different Mizaj.

**Conclusion:** Metabolomics has a potential role to establish a new approach to understanding the main concepts of PM such as Mizaj, classifications of diseases and effectiveness of traditional formula on humans with different Mizaj. Given that most of the research work is focused on herbal medicines and diseases and the important issue of temperament in human samples has done less work, it seems that in modernizing traditional medicine using new approaches such as metabolomics, the first step should be the basics of this medicine should be removed.

**Keywords:** Metabolomics, Persian Medicine, Mizaj, Temperament.

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## INTRODUCTION

The history of the use of traditional medicine dates back to the ancient human societies; they were even intermingled with religious beliefs in these societies (Khoomrung et al. 2017). Today, the traditional medicine are accepted among many parts of the world; for instance, about 80% of Africans still trust Traditional African Medicines (TAM) for a wide range of ailments. Also, the encouraging economic income in this field has attracted the attention of many countries and according to WHO it is believed that by 2030 Traditional Systems of Medicine can become a 115-billion-dollar business (Quansah and Karikari, 2016).

Among the various branches of traditional medicine, Persian Medicine (PM) and its exceptional physicians such as Avicenna (author of Canon) and Razes (author of Al- Havi) occupy a special place.

For a better understanding of PM and the reason why it is still accepted in modern times, we need to know its basic principles and philosophy.

The key to the introduction to PM is a topic known as "Natural Affairs". This topic consists of Elements (Arkan), Temperament (Mizaj), Humour (Akhlat), Organs (Aaza), Pneuma (Arwah), Faculties (Quwa), and Functions (Afaal). Among them, Elements, Temperament, and Humours are more important and have a cause and effect relationship

(Soltani et al. 2013).

The concept of Temperament is very crucial because the physician should find the primary temperament first and then identify the root of temperament imbalance to advise the appropriate therapeutic plan. “Mizaj” is defined as the unique temperament based on the specific humoral constitution of an individual. Any deviation from the normal temperament can result in illness. The recent emergence of “Personalized Medicine” also shows that the medical community is becoming interested in the concept of temperament.

Avicenna describes the Temperament as a state made from the interaction between the opposite qualities in an object and the emergence of uniform quality in every particle of that object (Abbasian et al. 2017). Other scholars such as Jorjani, Mohammad- Bin- Abdullah, and Aghili Khorasani present the same definition of Temperament. In other words, Mizaj determines all morphologic, physiologic and behavioral characteristics of every human being.

Every individual is born with a specific Temperament, which is called the Primary Temperament and can be allocated to one of these nine groups:

1) Warm 2) Cold 3) Moist 4) Dry 5) Warm and Moist 6) Warm and Dry 7) Cold and Moist 8) Cold and Dry 9) Balanced (Abbasian et al. 2017).

There are other signs known as “Tenfold signs” that are used to reveal the primary temperament:

1. Tactus or the quality of the skin on touch
2. Frame or body size, slimness or obesity
3. Hair and its quality
4. Color of the body
5. Size of body organs
6. Vulnerability to Coldness or Hotness or Dryness or Wetness
7. Sleep and Wakefulness
8. Quality of functions
9. Quality of body excretions such as stool, urine, and sweat
10. Mental and emotional state (Mojahedi et al., 2011).

One important point is the indexes of temperament determination. The indexes related to the four qualities are defined as a spectrum in such a way that one end of the spectrum shows one quality and the other end shows the opposite quality while the middle part shows the balanced state of the temperament. The personal temperament of any individual is located on a specific part of this spectrum and if the temperament crosses its specific place, it becomes a Dystemperament (Mojahedi et al. 2011).

Since our physical, emotional, and physiological characteristics change according to different stages of our lives and other influential factors, our primary temperament can transform to a new one known as the “Secondary temperament” (Abbasian et al., 2017). Therefore, it is an

important task for the physician to distinguish between the two and keep the body healthy or cure the disease.

Similar to the concept of temperament in PM, nowadays the physical and mental-emotional differences of individuals have become an interesting topic for researchers in the field of medicine leading to the emergence of Pharmacogenetics, Metabolomics, Nutrigenomics, Proteomics, and Genomics. They have become the stepping-stone for a new term in medicine known as “Personalized Medicine” (Mojahedi et al. 2011) (Rezaeizadeh et al. 2022).

Therefore, the concept of Temperament in PM can be an exciting subject for those scholars interested in Personalized Medicine. There are four explanations for understanding temperament in modern medicine: 1) Relating the coldness and hotness to plasma acidity 2) Hyperthyroidism and hypothyroidism 3) Renal function, water and electrolyte balance; those supporting this idea believe that accumulation of water and electrolytes in the body corresponds to wetness whereas their depletion corresponds to dryness 4) Neuroendocrine response; the supporters of this idea believe that individuals with hot temperament have a higher activity of sympathetic nervous system compared to individuals with cold temperament (Ahanchi and Saiedimehr, 2011).

It should be acknowledged that finding a common ground between views of traditional medicine and that of modern medicine is an obstacle. First, the holistic view and philosophy of traditional medicine may seem strange in the view of modern medicine. Also, a lack of sufficient indexes on the molecular level limits the use of traditional medicine in clinical practice (Khoomrung et al. 2017). In brief, many bioactive compounds in traditional medicine are still undetermined and mechanisms of action are not clear (Wang et al. 2017).

To solve these problems, we need to find a suitable branch of science for better understanding and modernizing of PM. Although in previous researches, a range of scientific fields such as thermodynamics, neurology, endocrinology, and energy metabolism have been tested for conceiving the concept of temperament, it is the metabolomics which is being increasingly used for identifying the diseases, their mechanisms, and biomarkers (Kuang et al. 2012). Metabolomics is the science of evaluation and measurement of small metabolites (less than 1500 Dalton) which are the result of environmental factors or imbalance of homeostasis. This term is sometimes used interchangeably with metabonomics. Metabolomics helps us understand the holistic concept of traditional medicine by analyzing the metabolic pathways in an organism (Chen et al., 2015). Quansah and Karikari mention that metabolomics can be used by two approaches of targeted and untargeted profiling; while the targeted approach targets a particular division of metabolites in a model, the untargeted method studies the complete metabolome. Therefore, untargeted metabolomics has become a valuable method to achieve a broad qualitative and quantitative assessment of herbal products (Quansah

and Karikari, 2016).

The goal of untargeted metabolomics is to study and measurement the whole metabolites (known and unknown metabolites without preconception), while targeted metabolomics, deals with the measurement of specific metabolites or focuses on one or more metabolic pathways by applying standards (Corcuera et al. 2009). In this review, we focus on untargeted methods and studies on traditional medicine.

There are two subsections in untargeted classification: metabolic fingerprinting and metabolic footprinting. Metabolic fingerprinting is the investigation and analysis of the collection of metabolites without any previous assumption (Dettmer and Hammock. 2004). Metabolic footprinting usually applies in microbiology and biotechnology researches and that's aim is the measurement of changes caused by organisms in laboratory culture media (Villas-Boas et al. 2005). Recently, traditional medicine has attracted special attention in the world which states that traditional medicine has the potential to complement and supplement modern medicine.

In this article, we will discuss the role of metabolism in advancing the understanding of the mechanism of traditional medicine and reviewing studies in this field.

## MATERIALS AND METHODS

In this review study, in an advanced search of papers published in English language from 2003 to 2020 with key words of “traditional medicine”, “temperament” and applications of new techniques such as “metabolomics” in this medicine, in the databases of PubMed, Medline, ScienceDirect, Cochrane, Embase is used.

## RESULT

China has been a frontrunner in terms of using Metabolomics in traditional medicine; table 1 presents a summary of studies conducted in China in this field. Most of published studies were animal study. Interestingly, some researches focused on the effects of traditional formula on the metabolomics.

Table 1: Literature review of the application of Metabolomics in Chinese Traditional Medicine

Row	Subject	Sample	Technique
1	Plasma metabolomics of depressed patients and treatment with Xiaoyaosan based on mass spectrometry technique(Lio et al. 2020)	Human plasma	GC-MS
2	Blood metabolism study on protection of residual renal function of hemodialysis patients by traditional Chinese medicine Kidney Flaccidity Compound (Hu et al. 2018)	Human plasma	UPLC
3	Exploring pathogenesis in subjects with subjective Tinnitus having kidney deficiency pattern in terms of Traditional Chinese Medicine based on serum metabolic profiles (Yong et al. 2018)	Human serum	UPLC-Q-TOF/MS
4	Anti-depressive effects of Kai-Xin-San on lipid metabolism in depressed patients and CUMS rats using metabolomic analysis (Zhou et al. 2020 )	Human plasma	UPLC-Q-TOF/MS
5	High-throughput metabolomics used to identify potential therapeutic targets of Guizhi Fuling Wan against endometriosis of cold coagulation and blood stasis (Wu et al. 2018)	Rat urine	UPLC-Q-TOF-MS with pattern recognition methods
6	Serum metabolomics strategy for understanding the therapeutic effects of Yin-Chen-Hao-Tang against Yanghuang syndrome (Liu et al. 2018)	Rat serum	UPLC-Q/TOF-MS techniques
7	Plasma metabonomics study of rheumatoid arthritis and its Chinese medicine subtypes by using liquid chromatography and gas chromatography coupled with mass spectrometry (Yan et al. 2012)	Human plasma	(LC-MS) and gas chromatography-mass spectrometry (GC-MS).
8	Chemical metabolomics for investigating the protective effectiveness of <i>Acanthopanax senticosus</i> Harms leaf against acute promyelocytic leukemia (Han et al. 2018)	Mice serum	UPLC-Q-TOF-HDMS
9	Characterization of Rheumatoid Arthritis Subtypes Using Symptom Profiles, Clinical Chemistry and Metabolomics Measurements (Wietmarschen et al. 2012)	Human plasma and urine	LC-MS method
10	Systems Biology Guided by Chinese Medicine Reveals New Markers for Sub-Typing Rheumatoid Arthritis Patients (Kailong et al. 2009)	Human plasma	gas chromatography/mass spectrometry
11	A combination of cecum microbiome and metabolome in CUMS depressed rats reveals the antidepressant mechanism of traditional Chinese medicines: A case study of Xiaoyaosan (Meng et al. 2012)	cecal samples of Rats	H-NMR

12	Metabonomics Research of the Four Properties in Traditional Chinese Medicine Based on UPLC-QTOF-MS System (Kuang et al. 2012)	Rat urine	UPLC-QTOF-MS
13	Application of metabolomics on diagnosis and treatment of patients with psoriasis in traditional Chinese medicine(Lu et al. 2014)	Human urine	HPLC
14	Metabolomic and lipidomic study of the protective effect of Chaihuang-Yishen formula on rats with diabetic nephropathy (Zhao et al. 2015)	Fresh tissue of rat kidney cortex	UPLC-TOF MS & GC-TOF MS
15	Plasma-metabolite-biomarkers for the therapeutic response in depressed patients by the traditional Chinese medicine formula Xiaoyaosan (Liu et al. 2015)	Human plasma	nuclear Magnetic Resonance ( <sup>1</sup> HNMR)
16	Effect of a traditional Chinese medicine prescription Quzhuotongbi decoction on hyperuricemia model rats studied by using serum metabolomics based on gas chromatography-mass spectrometry (Chen et al. 2015)	Rat serum	GC-MS
17	Serum metabolomics study of Traditional Chinese medicine formula intervention to polycystic ovary syndrome (Lu et al. 2016)	Human serum	UPLC LTQ-Orbitrap MS
18	Arachidonic acid metabolomic study of BPH in rats and the interventional effects of Zishen pill, a traditional Chinese medicine (Bian et al. 2016)	Rat plasma and urine	UHPLC- MS/MS
19	Thyroxine and reserpine-induced changes in metabolic profiles of rat urine and the therapeutic effect of Liu Wei Di Huang Wan (Wang et al. 2010)	Rat urine	UPLC-HDMS
20	Metabonomic study on “Kidney-Yang Deficiency syndrome” and intervention effects of Rhizoma Drynariae extracts(Lu et al. 2011)	Rat urine	UPLC-MS
21	NMR-spectroscopy-based metabonomic approach to the analysis of Siwutang, a novel prescription, treated blood deficiency in mice (Wang et al. 2010)	Rat serum	NMR
22	Metabonomic study of the syndrome of liver qi stagnation and spleen deficiency (Luo et al. 2007)	Rat blood	HNMR
23	Metabonomics study on the effects of the ginsenoside Rg3 in a $\beta$ -cyclodextrin-based formulation on tumor-bearing rats (Wang et al. 2008)	Rat urine	HPLC-ESI-MS
24	Effect of a traditional Chinese medicine preparation Xindi soft capsule on the rat model of acute blood stasis (Zhao et al. 2008)	Rat urine	UPLC-MS
25	Application of GC/MS-based metabonomic profiling in studying the lipid-regulating effects of Ginkgo biloba extract on diet-induced hyperlipidemia in rats (Zhang et al. 2009)	Rat urine	GC-MS
26	Metabonomic characterization of aging and investigation on the anti-aging effects of total flavones of Epimedium (Yan et al. 2009)	Rat urine	LC-MS
27	Metabonomics investigation of human urine after ingestion of green tea(Wai Siang et al. 2010)	Human urine	NMR & GC/time-of-flight MS
28	Metabonomics approach to determine metabolic differences between green tea and black tea consumption(Van Dorsten et al. 2006)	Human plasma and urine	GC&MS& Rapid common. MS

The results of researchers seeking the relationship between metabolomics and theory of Traditional Chinese Medicine (TCM) have led to identifying different profile metabolic characteristics in heat and cold pattern based on TCM (Yavari M. 2021) (Chunxiu et al. 2014). van Wietmarschen et al. have established some curative studies on metabolomics and subtypes of rheumatoid arthritis according to Chinese Medicine Theory (Yan et al. 2012), (Wietmarschen et al. 2012) (Kailong et al. 2009). For example, level of 11 acylcarnitines, DHEAS and CCP, glycolchenodeoxycholate, proline, saturated and

monounsaturated phosphatidylcholine were higher in hot versus cold ZHENG in patients with rheumatoid arthritis (Yan et al. 2012).

Considering concept of Mizaj in PM, some researchers have been trying to find a connection between different Mizaj and cellular and molecular indexes. A paper published by Dar et al. showed that level of alkaline phosphatase enzyme was different in individuals with hot-wet and cold-wet Mizaj (Farooq et al. 2011). Furthermore, a recent network-based study published by Rezadoost et al. on proteomics profiles of individuals with hot-wet and cold-dry temperament

showed that most detected proteins were different between two groups (Rezadoost *et al.* 2016). The results of this study encourage further investigations including metabolomics studies in different Mizaj groups.

Xijun Wang *et al.* indicate that metabolomics has the potential to find a new path for unlocking the function of traditional medicine through the modernization of old concepts and therefore decrease the gap between traditional medicine and modern medicine (Wang *et al.* 2011). Shi *et al.* mention that metabolomics, as an important part of the biologic system, makes it possible for us to quantitatively measure the metabolites of small molecules involved in metabolic pathways and therefore reflect the state of basal body metabolism (Shi *et al.* 2015).

It should be noted that tendency of Chinese researchers toward using metabolomics in Traditional Chinese Medicine is to such that they follow a new approach called “Chinmedomics” to updates syndromes and traditional formulae with the latest techniques (Zhang *et al.* 2019). Furthermore, potential application of metabolomics as a tool for evaluation of drug toxicity in TCM has been considered (Duan *et al.* 2018).

One of the main advantages of Metabolomics in the study of disease and drug treatment is that metabolic profiling can be done using biologic fluids (serum, plasma, saliva, urine, CSF,...) and the availability of plasma and urine makes it easy for us to conduct large scale studies (Wang *et al.* 2011). This has led to the rise of comprehensive databases such as the Human Metabolome Database (HMDB), which is updated each year with studies about metabolites and metabolic pathways (Wishart *et al.* 2018).

Metabolomics studies are based on a variety of analytical tools. The most useful analytical instruments are nuclear magnetic resonance (NMR) spectroscopy or hyphenated mass spectrometry such as GC-MS, LC-MS, and CE-MS. In Metabolomics studies, the researchers may choose a particular approach based on their objectives, facilities, and the abilities of their team. Each technique has its advantages and disadvantages (Farrokhi Yekta *et al.* 2017); for instance, Liquid Chromatography-Mass Spectrometry (LC-MS) has the advantage of better coverage of mass spectrum but it is a very costly technique (Mao *et al.* 2017).

We should know that metabonomics approaches produce very complex data; therefore, using multi-variable statistical tools to analyze and interpret the biological and chemical data is necessary. Among the chemometrics tools, Principal Component Analysis (PCA) and Orthogonal Projections to Latent Structures Discriminant Analysis (OPLS-DA) have been suggested for the categorization of four temperaments (Kuang *et al.* 2012).

Each of these steps has many concepts and procedures and sub-sections, that this review is not the place to discuss them; actually, Metabolomics is dynamic and rapidly evolving field of life science (Trivedi *et al.* 2017).

Despite the advancements in the research of metabolomics,

nevertheless, there are limitations in this area:

- a) Still, there are not enough techniques available for the whole range of metabolites.
- b) The huge amount of data is difficult for analysis.
- c) Using findings in Metabolomics along those of Genomics and Proteomics is not a focus for modern medicine (Quansah and Karikari, 2016).

### PM in the Regulatory and Statistical Sciences

The designing and analysis of the TCM clinical trials and their regulatory and statistical issues in the regulatory agencies for example the United States Food and Drug Administration (FDA) and pattern discovery, data mining and artificial intelligence methods based on the metabolic substances of herbal medicine are well studied [Chow, 2019] [Poon, 2014]. Some recent studies, use a new method for sample size estimation, MetSizeR approach [Nyamundanda *et al.*, 2013], for TCM researched with Metabolomics approach [Song, 2019] [Song, 2019] [Xiao, 2020]. In this regard, one of the the (T)PM directions for future is developing methods for designing, analysis and presenting the Clinical trials and research proposals with considering statistical and regulatory issues and using high-dimensional and high-throughput datasets.

### CONCLUSION

PM can be viewed from different angles: 1) The principles such as Temperament 2) The ailments which can be viewed under the concept of Distemperament. Herbal remedies in the form of single herbs or compound preparations because most of the researches in PM concern the herbal remedies or the ailments; we feel the necessity of modernizing the PM by novel techniques and explore the concepts such as Temperament by working on human samples. Since Metabolomics is very useful in finding the biomarkers and mechanisms of disease, it can explore a suitable structure for types of Temperament.

Metabolomics can help us to:

- Better understanding the concept of spectrum of Mizaj based on differentiation of metabolome in humans with different Mizaj.
- Understanding relationship between alteration in Mizaj and alteration in metabolome.
- Achieving to an index for quantitative evaluation of hotness, coldness, wetness and dryness of human beings with different Mizaj.
- Precise evaluation of distemperament according to alteration in metabolomics.
- Explanations of different subtypes of diseases according to PM.
- Evaluation and monitoring of the efficacy of herbal medicine and natural products on metabolomics of individuals with different Mizaj.

- Choosing the best drug therapy with higher efficacy and lower side effects based on the specific metabolome profile of every Mizaj.

It seems that some factors such as having research experts, available advanced tools, and cost-effective as well as effective analysis methods are limitations and difficulties of applying metabolomics in research and preclinical works. Although the science of Metabolomics can be of great help for modernizing PM, we should stress that using this method should be confined to understand the simple mechanism of this medicine, not to extinguish the ancient philosophy of PM. Regarding limited research works in the field of metabolomics and PM.

It is strongly suggested to do further metabolomics researches in order to achieve a common pathway between PM and modern medicine.

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