BIOCHEMISTRY AND MOLECULAR BIOLOGY IN THE POST GENOMIC ERA

# METABOLOMICS *And* CLINICAL APPROACH

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# Contents

# Chapter 24

# Metabolomics in Biomarker Identification for Cardiovascular Diseases

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## Abstract

Metabolomics is the systematic analysis of the particular chemical fingerprints of small molecules or metabolite profiles which are associated with a different cellular metabolic process in a cell, organ, or organism. Events in a cell are not described completely by messenger RNA gene expression data and proteomic analyses, but metabolic profiling supplies direct and indirect physiological insights, which can possibly be measurable in a broad range of biospecimens. Even though not specific to cardiac conditions, identification, confirmation, clinical validation, and bedside tests are a biomarker exploration path to translate metabolomics into cardiovascular biomarkers. Technological progress in metabolomic tools (such as nuclear magnetic resonance spectroscopy and mass spectrometry) and more complicated bioinformatics and analytical techniques help to evaluate low- molecular-weight metabolites in biospecimens and ultimately supply a unique insight into determined and novel metabolic pathways. Systematic metabolomics can provide physiological knowledge of cardiovascular disease states in addition to traditional profiling and can include the definition of metabolic reactions of an individual or population to therapeutic interventions or environmental exposures.

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## Introduction

A severe popular health challenge and the main cause of death has been cardiovascular diseases (CVDs) (D'Agostino et al., 2000; Kordalewska & Markuszewski, 2015). According to the World Health Organization, CVD is responsible for 17.9 million deaths in 2019 which is 32% of global deaths (World Health Organization (WHO), 2009). Future predictions show that CVDs could reach 23.3 million deaths in 2030 (Mathers & Loncar, 2006). For the European Union, the estimated number of the costs per year is 196 billion euros which include 54% direct healthcare, 24% performance deficits and 22% casual nursing of patients with CVD (Nichols et al., 2012).

Complications of the blood vessels and heart, which is a result of atherosclerosis, cause CVDs and are mostly diagnosed in old age in both men and women. These groups of diseases contain infarction, cardiogenic shock, and myocardial ischemia, atherosclerosis, anthracycline-induced cardiotoxicity, atrial fibrillation heart failure, ischemic cardiomyopathy, non-ischemic cardiomyopathy, coronary artery disease (CAD). Early diagnosis and intervention procedure will decrease the prevalence of CVD.

While many risk factors are responsible for CVDs, such as smoking, gender, renal failure, lack of nutritious dietary, overweight as a result of less body movements, cholesterol, increased blood pressure, and hyperglycemia, the pathological mechanism fundamental to most CVDs are still not fully known (Hackam & Anand, 2003; Kordalewska & Markuszewski, 2015; Libby, 2002; P. W. Wilson et al., 1998; P. W. F. Wilson, 2008). Moreover, in addition to popular risk factors for CVD, it is mostly expected that a patient with CVD could have another CVD as time progresses (Hunt et al., 2013; World Health Organization (WHO), 2007). Furthermore, one of the major problems for CVD patients is that signs of the diseases do not appear early in the progression of the disease. Actually, plaque formation will be formed during asymptomatic processes, which will build up silent but growing tissue damage (Barderas et al., 2011). When atheroma plaques subsequently tear, an atherothrombotic event arises due to the discharge of highly thrombogenic material (Barderas et al., 2011). When the metabolism is considered, the heart is a very significant part of the human being. Therefore, complications in the cardiac energy metabolism cause many cardiovascular diseases (Müller et al.,

2021; van Bilsen et al., 2009). Moreover, cardiac function and myocardial metabolism can be affected by the pathogenesis of cardiovascular disease. Cardiovascular diseases also induce changes in substrate metabolism that result in distinct changes in the patient's metabolic profile (Nascimben et al., 1996; Smith et al., 2006).

Depending on these events, clinical intervention and early diagnosis of CVDs require the discovery of original and practically important biomarkers, which are used alone or together with existing ones (Barderas et al., 2011). When evaluating particular responses to determine standard risk factors, a mixture of existing biomarkers will only contribute slightly (T. J. Wang et al., 2006). Thus, there is considerable interest in exploring and operating new biomarkers, which will help decide the most useful from the current list and diagnosing people with the potential to be caught in CVDs (Barderas et al., 2011). Especially, if the used biomarkers predict the risk of rupture, it will help patients receive pharmacological treatment in time and will create a protective lifestyle. The investigation and innovation of biomarkers for developments in outpatient and inpatient care adds a new dimension to the landscape of CVD. The improvements in "-omics" technologies (genomics, transcriptomics, proteomics, and metabolomics), which have fast, sensitive and robust tools, have made it easier to examine these small molecules in cardiovascular diseases.

## Metabolomics Technologies

Tiny particles, which are responsible for common responses related to metabolism and which are necessary to continue the sustenance, advancement, and regular action of a cell, are called metabolites. Moreover, metabolome, derived from the word genomics, is defined as the entire group of metabolites, which can be found in a living thing and its organs (Fiehn et al., 2000; Oliver et al., 1998) or the whole metabolites in a cell (Tweeddale et al., 1998). Thus, usage regarding investigative procedures on determining and measuring whole metabolites in the body, along with observing the differences in the metabolome of the cell cultivation, tissue model, or a biofluid as a result of perturbation are called as metabolomics and metabonomics (Fiehn et al., 2000; Nicholson et al., 1999).

Cardiovascular diseases cause pathophysiological molecular, cellular and functional changes, which can be better understood with the help of new "omics" tools (genomics, transcriptomics, proteomics, and metabolomics)

(Roberts & Gerszten, 2013). The excessive number of these small molecules in liquid body substances (saliva, urine, blood) and breathing exhalation can be measured by the use of modern metabolomics technologies (Müller et al., 2021; Shah et al., 2012). This information might be used as predictive and characteristic tools to analyze early specific alterations during the beginning and progression of cardiovascular disease (Müller et al., 2021). For that reason, metabolomics is projected as a valuable means of providing additional insight into pathologic physiology in CVDs along with increasing practitioners' knowledge of pathogenesis of CVDs (Müller et al., 2021).

One of the interesting characteristics in the area of metabolites is that the number of metabolites in the body is slightly small (≈7000) when compared to the approximate number of transcripts (100000), genes (25000), and proteins (1000000) (Shah et al., 2012). Nonetheless, concentration ranges of metabolites are very wide, and metabolites display significant chemical diversity. Therefore, it is impossible to measure the metabolites in the human metabolome with the use of currently available instruments in an individual analysis (Shah et al., 2012). Alternatively, metabolome analysis can be performed with several analytical strategies (Dunn et al., 2005), such as nuclear magnetic resonance (NMR) (Nicholson & Wilson, 2003), mass spectrometry (MS), Fourier transformation infrared spectroscopy (FT-IR) (Harrigan et al., 2004; Johnson et al., 2004), along with analysis methods for example capillary electrophoresis (CE), gas chromatography (GC), or high performance liquid chromatography (HPLC) (Barderas et al., 2011). The metabolomics study includes various research approaches such as metabolic fingerprinting, metabolic profiling, and metabolic foot printing (Kordalewska & Markuszewski, 2015). Metabolic profiling is an illustration of the targeted approach and in this technique metabolites, which have similar physical characteristics such as carbohydrates, amino acids, grouped in a specific biological approach such as purine metabolism and glycolysis are discovered. A specific section of the metabolome composition can be learned better qualitatively and quantitatively with the help of the data, which is gathered with this strategy (Kordalewska & Markuszewski, 2015; Patti et al., 2012). In the case of the metabolic fingerprinting method, which is an example of an untargeted approach, metabolites are investigated without previous information. Therefore, the whole metabolome might be discovered. Metabolites' levels vary under precise systems' conditions by showing a specific pattern, which can be described by this method. The metabolic footprinting approach is used mostly in molecular biology and microbiological studies. Metabolites, which are secreted into the culture medium by cells and

microorganisms, could be recognized in these experiments (Kordalewska & Markuszewski, 2015; Patti et al., 2012).

## Metabolomics in Cardiovascular Diseases (CVDs)

Metabolomics is being performed to examine various forms of causes of CVDs such as diabetes mellitus, fatness, and metabolic syndrome (Dumas et al., 2006; Faber et al., 2007; T. J. Wang et al., 2011). Mostly, it has focused on analyzing markers and explaining the natural history linked with various common pathologies in CVDs. These have extended markers for cardiogenic shock (Nicholls et al., 2007), myocardial ischemia (Lewis et al., 2008, 2010; Sabatine et al., 2005), risk of developing atherosclerosis or future cardiovascular events (Tang et al., 2009; Z. Wang et al., 2009b, 2011), atrial fibrillation (Mayr et al., 2008), risk of developing diabetes mellitus (T. J. Wang et al., 2011), chemotherapy-induced cardiotoxicity (Andreadou et al., 2009) and pulmonary hypertension related to advanced heart failure (Shao et al., 2012). These are some of the examples of cardiovascular events showing that metabolomic data metabolomic data have a wide application in the control of CVDs (Senn et al., 2012).

### Metabolomics in Myocardial Ischemia, Cardiogenic Shock, and Infarction

The possible discovery of metabolic imbalance, which may occur during or after myocardial ischemia, can become an encouraging diagnostic instrument. In the work of Sabatine et al., (Sabatine et al., 2005), LC-MS (Liquid chromatography mass spectrometry), an untargeted approach, was used to examine metabolic profiling of myocardial ischemia. In their work, specimens of patients with and without myocardial ischemia were collected to test the difference of workout in patients. Therefore, differences in small molecules (metabolite) before and after to stress examination helped them determine the possible signs (biomarkers) of coronary ischemia. The levels of 6 metabolites changed and were recognized as inducible ischemia with a validated risk score. γ-aminobutyric acid, citric acid, uric acid, and several more metabolites were determined at an abnormal level. It was concluded that studies in metabolomics will be helpful in diagnostic studies and eventually will analyze

new goals for therapeutic intervention. Apparently, cardiovascular metabolic changes, which can be discovered with serial blood sampling, occur after exercise (Senn et al., 2012).

To continue further ischemia, Lewis et al., (Lewis et al., 2008) worked on an artificial infarction design with environment of a septal ablation for obstructive hypertrophic cardiomyopathy to determine the momentary diversities metabolomic profiling throughout the progression of myocardial infarction. The interesting results of their work showed that the plasma marker of threonine, aconitic acid, hypoxanthine, and trimethylamine-N-oxide (TMAO) discriminated among with infarcted and with coronary angiography patients.

The array of disorders in the body can also help to understand the level of the disease. Intense heart attack (acute myocardial infarction (AMI)), which is made difficult by cardiogenic shock, causes serious morbidity and mortality rates in spite of tough therapy techniques such as revascularization (Hochman et al., 1999; Hochman et al., 2001). Nitric oxide (NO) has useful impacts containing coronary vasodilation and additional resting in the bed. Moreover, NO has various harmful effects, such as, lowering the ability of heart to contract, thus it can perform an essential duty in the hypotension and reduced heart functionality, which describe cardiogenic shock. It has been hypothesized that NO may participate in the pathogenesis of shocks in the heart, depending on the idea that stimulation in the inflammatory cells may cause isoforms of NO synthesize (Hochman, 2003). People with and without coronary artery disease are studied in the work of Nicholls et al., (Nicholls et al., 2007), and comparisons of metabolic profiles were made. Thus, patients after AMI are complicated by cardiogenic shock. In their results, it was found that the increased amount of the NO synthesize blockage, asymmetric dimethylarginine (ADMA) presents to be a free predictor of death. On the other hand, a future study of that group could not find a death advantage with inhibition of therapeutic NO synthase by L-NG-monomethyl arginine (TRIUMPH Investigators et al., 2007).

## Metabolomics in Atherosclerosis

The metabolic profile that occurred during atherosclerosis should be examined because approximately all CVDs have atherosclerosis as an underlying process. The Multi-Ethnic Study of Atherosclerosis was started in 2000 in the US and that project also included the metabolomics approach (Bild et al.,

2002; MESA, 2022). In one of the studies, the relation between inflammation in the immune system caused by fat having a dangerous effect on atherosclerosis and plasma phospholipid polyunsaturated fatty acids degrees of was examined (Steffen et al., 2012).

Another broad investigation was occurred in 2012 to improve the identification of atherosclerosis before the infirmary stage. Tyrosine, glutamine, and docosahexaenoic acid were found to be a possible indicator of atherosclerosis growth as a result of serum metabolic profile analysis by using the NMR technique (Vorkas et al., 2015). Chen et al., (Chen et al., 2010) the used GC–MS method along progressive bioinformatics devices and 1-monolinoleoylglycerol, stearate, and palmitate were found as a possible plasma biomarkers of the disease.

The metabolic phenotype of the atherosclerotic plaque was examined and a comparison of the metabolome arrangement of the intima tissue along with atherosclerotic plaque tissue of people with carotid or femoral endarterectomy was obtained. UHPLC–MS (Ultra-high performance liquid chromatography-Mass spectrometer) method was applied to analyze tissue extracts. The degrees of acylcarnitines were different, which demonstrated changes in β-oxidation operation in the atherosclerotic plaque tissue. Also, phosphatidylethanoleamine-ceramides were recommended to be possible biomarkers of atherosclerosis pathogenesis (Vorkas et al., 2015).

## Metabolomics in Heart Failure

Zhang et al., (Zhang et al., 2009) analyzed the sequence of myocardial infarction caused by isoproterenol in rats in metabolism. They performed UHPLC–TOF/MS (ultra-high performance liquid chromatography time-of-flight/ mass spectrometry) examination and found differences in the degrees of 13 plasma lipids (phospholipids and fatty acids) in rats with activated myocardial infarction (heart attack) as distinguished from healthy people.

Another study on heart attack was performed in pigs and in the control zone, the “at-risk” regions and the necrotic districts of the hearts were imposed on resection. As a result of the NMR-based metabolic fingerprinting approach, the quantity of small metabolites decreased and the lipid signs were found in necrotic cells (Barba et al., 2007).

The operation of the GC–TOF/MS (Gas Chromatography–Time-of-Flight/Mass Spectrometry) method showed the existence of possible serum biomarkers in the diagnosis of heart failure. As a result of the comparison

between patients and healthy individuals, 2-hydroxy 2-methylpropanoicacid, pseudouridine, 2-oxoglutarate, 2,4,6- trihydroxypyrimidine and erythritol were different (Dunn et al., 2007).

The application of the LC–QqQ/MS (liquid chromatographic-triple quadrupole tandem mass spectrometry) technique was used to perform metabolomic analyses in plasma and it was found that asymmetrical dimethylarginine, N-mono-methylarginine, and symmetrical dimethylarginine were possible dangerous predictors of incident cardiac occurrences (Z. Wang et al., 2009a).

The molecular process of acute coronary syndrome was analyzed using the GC–MS technique with the help of plasma metabolic fingerprinting. Comparison was made between the metabolic characterization of 9 samples from acute coronary syndrome patients, ten examples of constant atherosclerosis patients, and ten examples of patients in good health. As a result of the comparison between acute coronary syndrome and healthy individuals, aspartic acid, 4-hydroxyproline, citric acid, and fructose levels decreased while glucose, urea, lactate, and valine amounts enhanced. The partial least squares discriminant examination (PLS-DA) was performed to discover the changes in metabolome composition and, as a result, the groups were classified as excellent. The categorization error was 5.3% for sick people with acute coronary syndrome and 0% for controls and patients with stable atherosclerosis (Vallejo et al., 2009).

## Conclusion

The scientific community has a growing interest in the metabolomics, which is one of the most commonly used omic-related sciences, over the last decade. A mixture of different sensitive analytical techniques and advanced chemometric tools are needed to be used in metabolomics analyses. The molecular processes underlying different diseases can be explained and understood better with the help of metabolomics application. As a result of acquired knowledge, determining the reasons behind diseases, optimizing the cure, and choosing particular characteristic biomarkers can be explained easily. When diseases progress asymptomatically or without particular diagnostic biomarkers, the operation of metabolomics approach becomes more important. These metabolomics analyses have a difficulty of excluding the several effects, for example, age, dietary, used pharmacotherapy and additional environmental aspects influencing differences in the metabolome.

The composition of the metabolites should change as a result of the development of disease particularly. In the case of untargeted analysis, knowledge on organisms' metabolome structure is provided. Nevertheless, confirmation of possible disease markers can only be provided by the quantitatively targeting the analysis of selected metabolites. These compounds will gain a diagnostic and prognostic power by the measurement of them in a large-scale population along with parallel validation. To summarize, the operation of metabolomics in biomedical research can develop the advancement in pharmaceutical studies. The work reviewed in this chapter demonstrated that there has been an increasing concern about the use of metabolomics in CVDs. The likelihood of utilizing various example classes such as urine, tissue, blood, and breath with a concurrent operation of particular analytic methods may supply the inclusion of all metabolites exist in the body. The above cited works presents cases in which the metabolomic path is becoming more developed into an effective instrument in the CVDs analysis.

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