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Metabolomics in cardiovascular diseases – current state of knowledge

Badania metabolomiczne w chorobach sercowo-naczyniowych – aktualny stan wiedzy

Jakub Szost, Grzegorz Raczak, Ludmiła Daniłowicz-Szymanowicz
Department of Cardiology and Electrotherapy Medical University of Gdańsk

Abstract

Metabolomics is a field of systems biology that deals with the evaluation of small particles called metabolites. Over the last two decades there has been a dynamic increase in metabolic research in medicine, also in cardiology. A number of metabolites in specific cardiac diseases and their potential clinical significance were described. Metabolic studies help to understand disease processes at the cellular level and offer the potential to create personalized treatment. However, the lack of standardization of metabolic studies, the potential impact of external factors and the use of complex laboratory methods limit their application in everyday practice. Despite these limitations, research in the field of metabolomics remains an interesting direction of scientific development, a method allowing to learn about metabolic pathways in specific diseases and a potential source of new cardiac biomarkers. *Geriatria 2020; 14: 70-75.*

Keywords: metabolomics, metabolite, systems biology, molecular biology, cardiovascular disease

Streszczenie

Metabolomika jest dziedziną biologii systemowej, która zajmuje się oceną małych cząsteczek zwanych metabolitami. W ciągu ostatnich dwóch dekad nastąpił dynamiczny wzrost badań metabolicznych w medycynie, również w kardiologii. Opisano szereg metabolitów w poszczególnych chorobach serca i ich potencjalne znaczenie kliniczne. Badania metabolomiczne pomagają zrozumieć procesy chorobowe na poziomie komórkowym i dają potencjał do stworzenia spersonalizowanego leczenia. Brak standaryzacji badań metabolicznych, potencjalny wpływ czynników zewnętrznych oraz zastosowanie złożonych metod laboratoryjnych ogranicza jednak ich zastosowanie w codziennej praktyce. Pomimo tych ograniczeń, badania w dziedzinie metabolomiki pozostają ciekawym kierunkiem rozwoju naukowego, metodą pozwalającą na poznanie szlaków metabolicznych w poszczególnych chorobach serca oraz potencjalnym źródłem nowych biomarkerów kardiologicznych. *Geriatria 2020; 14: 70-75.*

Słowa kluczowe: metabolomika, metabolit, biologia systemowa, biologia molekularna, choroby sercowo-naczyniowe

Introduction

Metabolomics is a relatively new field of systems biology, which deals with the study of metabolites. Metabolomics is a fast-growing scientific field, willingly used in medical research, e.g. oncology, neurology, or psychiatry. The fast development of research using metabolic analysis takes place in the last two decades. More than 100,000 metabolites are currently identified in the human body [1]. Information about the current state of knowledge is available in online databases (HMDB, METLIN, MMCD). This study aimed to present the literature reviewer concerning the usefulness of metabolomics in cardiovascular diseases and tried to explain some of the available cardiology data and their potential clinical significance.

The place of metabolomics in systems biology

Metabolites are low weight molecules (<1.5kDa) being products or intermediate products of biological processes occurring at the cellular level. They include carbohydrates, amino acids, organic acids, nucleotides,

fatty acids, and steroids [2]. The biological material used for metabolic analyses is relatively easily accessible. Most often for analysis, the following are taken: blood serum, plasma, urine, saliva, cerebrospinal fluid, and tissue fragments after their preparation.

The collection of all metabolites in a particular organism, tissue, or cell is called a metabolome. The metabolome is a concentration of a very large number of molecules with different concentrations and characteristics in a tested sample. It is a dynamic collection, susceptible to changes taking place both inside the cell and to eternal factors, such as the effects of drugs, diet changes, or stress [3]. This all adds up the difficulty of data analysis and the need to use advanced mathematical and statistical techniques to process data. It seems that the knowledge of metabolic processes in combination with analysis of proteome (protein collection), transcriptome (RNA collection) and genome (gene collection) will allow a better understanding of disease processes in the human body, the detection of new biomarkers and will be the basis for the introduction of individualized treatment. Figure 1 presents the place of metabolomics in systems biology [4].

Metabolomics uses modern analytical and chemometric techniques. The basic analytical tools of metabolomics are: liquid chromatography combined with tandem mass spectrometry (LC-MS/MS), gas chromatography combined with mass spectrometry (GC-MS), gas chromatography combined with tandem mass spectrometry (GC-MS/MS), tandem Fourier transform ion cyclotron resonance mass spectrometry and tandem mass spectrometry (FT ICR MS/MS) and nuclear magnetic resonance spectroscopy (NMR) [5].

Clinical application of metabolomics

We distinguish three different research strategies in metabolic analysis: targeted metabolic analysis, metabolic profiling, and metabolic fingerprinting.

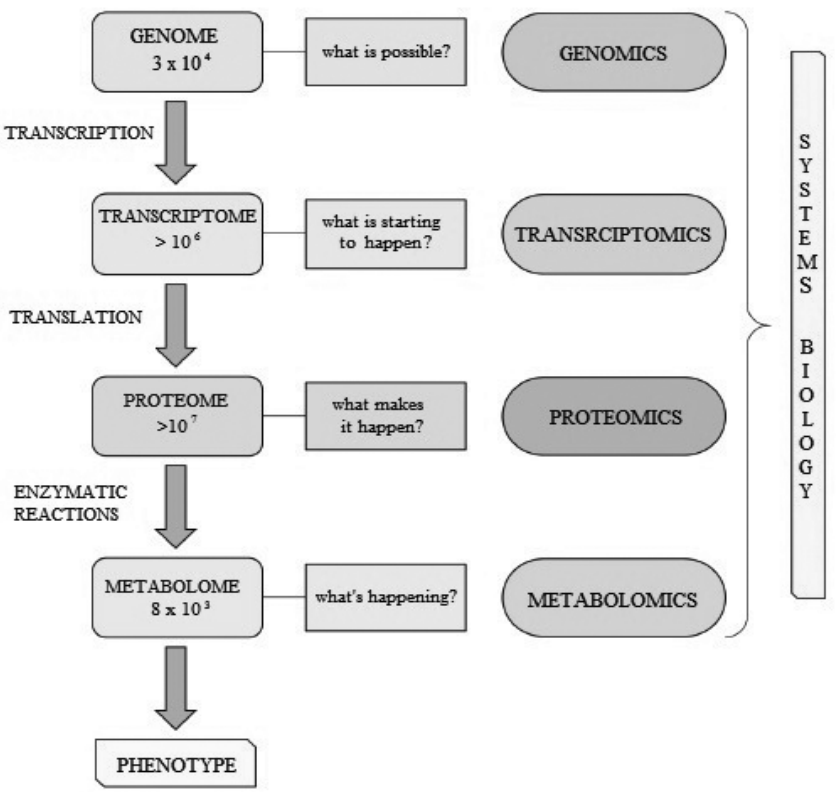

Figure 1. Based on article "New Opportunities from the Cancer Metabolome O. A. Aboud, R. H. Weiss, Clinical Chemistry, Volume 59, January 2013"

Targeted metabolic analysis is the determination of one or more specific metabolites and the possibility to measure them quantitatively. The compounds to be determined have already known metabolic pathways or are associated with a specific organism's reaction. This applies, for example, to the screening test. Metabolic profiling is a non-targeted study allowing a quantitative assessment. A study consisting of the analysis of groups with similar chemical properties or taking part jointly in a specific metabolic pathway (e.g. citric acid cycle). This approach allows for a better understanding of intracellular processes and creates new research hypotheses. Metabolic fingerprinting consists of an overall assessment of the metabolites from a given sample without a precise quantitative assessment and analysis of individual metabolites or their pathways. It is mainly used to classify samples e.g. comparison of the metabolic profile of the patient and healthy samples [4,5].

Ischemic heart disease

It is estimated that in most European countries, coronary heart disease is found in 20,000-40,000 people per million inhabitants. Population studies have shown that the incidence of ischemic heart disease increases with age and affects twice as many men as women [6]. Myocardial ischemia caused by limited blood supply by constricted atherosclerotic coronary arteries, such as their closure in the acute phase of myocardial infarction, induces several metabolic processes at the cellular level. These changes are mainly due to the transition to anaerobic glycolysis and increased oxidation of free fatty acids (FFA) to ensure adequate ATP levels. Besides, coronary reperfusion in the acute phase of myocardial infarction causes the reflow of oxidized and glucose-rich blood which leads to the formation of reactive oxygen species (ROS) in mitochondria. Understanding the processes taking place in tissues affected by ischemia-reperfusion injury (IRI) may allow us to find methods to prevent it [9,10].

Substrates for anaerobic and aerobic metabolism (glucose, free fatty acids, ketone bodies, pyruvate, lactate, leucine, and glutamate) – significantly lower levels of these metabolites (significantly higher pyruvate levels) were found in blood samples of the patient undergoing cardiac surgery. The study included 37 patients undergoing cardiac surgery using extracorporeal circulation. The metabolic analysis was performed on peripheral arterial blood samples and venous blood samples from the coronary sinus. In the samples subjected to reperfusion damage during cardiac surgery, the transition from aerobic to anaerobic glycolysis was observed. Besides, it was observed that patients with ischemic heart disease had a reduced baseline production of fatty acids and ketones compared to the control group (patients undergoing cardiac surgery for other reasons than coronary artery disease) [7].

Ketoglutarate, a metabolite included in the citric acid cycle; citrulline and arginine butyrate included in the urea cycle (which provides substrates for citric acid cycle) – similarly to the previous study, in subjects with induced ischemia, significantly lower levels of the mentioned above metabolites in collected blood samples were observed. The cited study used transient ischemia during the treadmill exercise test and the SPECT exercise test [8].

Succinate – an organic compound from the group of carboxylic acids. Is an intermediate product in the citric acid cycle. In the animal model study, an accumulation of succinate was found in myocytes of mice subjected to acute ischemia [9]. In addition, at the time of reperfusion, rapid oxidation of succinate (by succinate dehydrogenase, SDH) was observed, which fueled the production of reactive oxygen species in mitochondria. Therefore, inhibition of succinate accumulation may be one of the methods to prevent ischemia-reperfusion injury, also on the human model.

Hydrogen peroxide – one of the reactive oxygen species. The metabolite was determined in plasma of patients diagnosed for coronary artery disease and subjected to exercise SPECT imaging (dipyridamole test) [10]. In patients with positive SPECT results, the concentration of hydrogen peroxide was significantly higher in tested samples. The study shows that even transient myocardial ischemia causes the release of reactive oxygen species, which can cause premature myocyte death due to apoptosis.

Lipoxin A4 and leukotrienes – are lipoxygenase products and vasoactive agents Leukotrienes increase vascular permeability, cause the migration of leukocytes to the arterial wall, promote the formation of clots and the adverse phenomenon of vessel remodeling. The study analyzed plasma taken from the coronary artery from patients undergoing percutaneous transluminal coronary angioplasty (PTCA) [11]. There was a significant increase in the concentration of lipoxygenase products in plasma immediately after the PTCA procedure. These metabolites may, therefore, be a predisposing

factor for occlusion or accelerated stent restenosis and are a potential point for further metabolic studies.

Heart failure

The frequency of heart failure in the European population is estimated at 0,4-2%, which means that around 6,5-10 million Europeans are affected. It is estimated that 0,8-1 million people in Poland suffer from heart failure [12]. It is often even used to say that we are currently dealing with "an epidemic of heart failure". In a failing heart muscle, ATP levels are 30% lower compared to a healthy heart [13]. For a long time, there has been a concept that assumes that there is a defect in the heart muscle that allows for adequate ATP production. Understanding the metabolic pathways and potential metabolic defects may receive more effective treatment of patients with heart failure.

Long-chain acyclocarnitine (LCAC) – the study of these metabolites was carried out on a large group of patients (almost 10,000 patients) undergoing cardiac catheterization due to heart failure. The material for metabolic analysis was blood taken from the femoral artery. Elevated levels of this metabolite were found in the group of patients diagnosed with heart failure with preserved ejection fraction (HFpEF) and heart failure with reduced ejection fraction (HFrEF), however, in the group of HFrEF the had a significantly higher level [14]. A close inverse correlation was found with LCAC level and left ventricular ejection fraction (LVEF). High levels of this metabolite indicate a common metabolic pathway in both HFpEF and HFrEF groups and impaired oxidation of free fatty acids (ineffective beta-oxidation) in both groups. In a further study on long-chain acyclocarnitine (LCAC), 41 patients with severe heart failure who were treated with a left ventricular assist device (LVAD) implantation were studied. The use of LVAD significantly reduced the level of LCAC in the tested blood samples. A close correlation between LCAC and mortality in this group was also observed [15].

Beta-hydroxybutyrate – a metabolite belonging to ketone bodies. In the study on 15 patients with non-ischemic cardiomyopathy were compared to the 20-person control group [16]. Increased use of beta--hydroxybutyrate in the heart muscle of patients with end-stage heart failure was found. These results suggest increased use of ketone bodies as an energy substrate in the final stage of heart failure.

Alanine, arginine, leucine, isoleucine, methionine, serine, glutamate – these amino acids have been shown to increase significantly in patients with coronary artery disease and heart failure with LVEF <40% undergoing an exercise test [17]. The analysis of metabolites was performed from blood plasma before and after the exercise test. Changes in the level of these amino acids indicate increased protein breakdown in patients with heart failure (proteolysis). Also, the study also showed a decrease in the process of glycolysis, glucose uptake in peripheral tissues ad increased concentration oxidation of free fatty acids. These changes in metabolism may result in form elevated levels of norepinephrine and glucagon in this group of patients and lead to the development of insulin resistance.

Pyridonoline and deoxypyridinoline – metabolites were determined from daily urine collection in patients with severe heart failure (NYHA III and IV), also those during therapy with catecholamines and LVAD implantation [18]. Elevated levels of both these metabolites were detected concerning the reference values. These molecules are also considered as biomarkers of osteopenia or osteoporosis in this group of patients.

Atrial fibrillation

Atrial fibrillation is the most common cardiac arrhythmia, which increases in frequency with age. Currently, the prevalence of atrial fibrillation is estimated at 2.9% of the adult population. In the 60-69 age group, 4.2% of the population is affected, in the 70-79 age group 9.7% and in the 80-89 age group as much as 13.4% [19]. The influence of atrial fibrillation on increased mortality, increased risk of stroke, dementia, and the development of heart failure has been proven [20]. Perhaps metabolic analysis may allow us to know the mechanism of arrhythmia formation and give a chance for its more effective treatment.

Glucose, beta-hydroxybutyrate, acetate – decreased concentration of these metabolites was observed in the group of patients undergoing CABG or valvular surgery who had atrial fibrillation during the perioperative period [21]. The material for the study was a fragment of the atrial appendage taken during the procedure. This study confirms the observation on the animal model where it was found that inhibition of glycolysis predisposes to atrial fibrillation.

AMP, hypoxanthin, xanthin – products of high--energy phosphate decay. In the animal model study (homogenate of the goat atrial fragment was collected),

high levels of these metabolites were found, resulting from an increased need for high-energy compounds in case of persistent atrial fibrillation [22]. A similar decrease in phosphocreatine (high energy phosphate) was observed.

Betaine, D-glutamic acid, glycerophospholine, and L-valine – the higher concentration of these metabolites was observed in the group of patients with atrial fibrillation (both in the taken samples from blood and tissue) [23]. The study compared metabolites obtained from both atrial tissue homogenate and blood collected during cardiac surgery. They obtained 30 samples with atrial fragments from patients with AF and 30 samples from patients without AF history. The best potential biomarker for atrial fibrillation detection in this study was D-glutamic acid.

Uridine, pseudouridine – it has been observed that high levels of these metabolites in collected blood samples and its correlate with the risk of atrial fibrillation. The study was performed on a large group of patients (approximately 2000 patients) [24].

Hypertrophic cardiomyopathy

Hypertrophic cardiomyopathy occurs in 0,2% of the population, with the same frequency in both genders and affects patients of all ages [25]. High patient mortality (1-2% per year) makes special efforts to learn about the pathogenesis of this disease.

Aconitic acid, threonine, trimethylamine oxide – early biomarkers of myocardial damage were searched for in patients with hypertrophic cardiomyopathy undergoing alcohol ablation [26]. Metabolites showed rapid growth (already after 10 minutes) induced by ischemia and may be used as an early marker of myocardial infarction.

BCAA (branched chain amino acids), triglycerides, and phospholipids – elevated concentrations of these metabolites were found in blood samples in patients with hypertrophic cardiomyopathy compared to the control group [27]. In study 53 examined patients were previously diagnosed as carriers of the MYBPC3-Q1061X gene, and 34 of them had confirmed hypertrophic cardiomyopathy.

Acyclocarnitine – accumulation of this metabolite was observed in patients with left ventricular hypertrophy regardless of initial heart disease. The accumulation of the metabolite is related to the inhibition of beta-oxidation of fatty acids. The study involved the collection of venous and arterial blood samples during cardiac catheterization [28]. Two subgroups of patients with hypertrophic cardiomyopathy (35 patients) and severe aortic stenosis (36 patients) were analyzed compared to the control group.

Conclusions

Metabolic studies are a promising medical research method, also in cardiology. However, due to dynamic changes in the metabolism, the influence of external factors, and lack of standardization in the performance of metabolic studies, they are not used in everyday practice. Metabolic studies, however, give greater insight into basic metabolic processes and changes occurring at the cellular level in specific disease entities. Such a look allows us to discover new metabolic pathways and gives a chance to detect new useful biomarkers. Publicly available databases and medical reports allow for a quick exchange of data and experiences between researchers. This, in turn, translates into an increasingly better understanding of the issues of metabolomics and the rapid development of this scientific field. Perhaps in the future, metabolic research will serve to create an individualized treatment and further medical breakthroughs.

Conflict of interest
None

Correspondence address
Jakub Szost
II Department of Cardiology
Medical University of Gdańsk
7, Dębinki St.; 80-211, Gdańsk, Poland
(+48 58) 349 39 10
34804@gumed.edu.pl

Piśmiennictwo/References

1. Wishart DS, Tzur D, Knox C, et al. HMDB the Human Metabolome Database. Nucleic Acids Res 2007;35:521-6.
2. Lewis GD, Asnani A, Gerszten RE. Application of Metabolomics to Cardiovascular Biomarker and Pathway Discovery. J Am Coll Cardiol. 2008 July 8; 2(2): 17-23.
3. Johnson CH, Gonzalez FJ. Challenges and Opportunities of Metabolomics. J Cell Physiol. 2012 August; 227(8):2975-81.
4. Klupczyńska A, Dereziński P, Kokot ZJ. Metabolomics in medical sciences – trends, challenges and perspectives. Acta Pol Pharm Drug Res. 2015;72(4):629-41.
5. Fiehn O. Combining genomics, metabolome analysis, and biochemical modelling to understand metabolic networks. Comp Funct Genomics. 2001;2(3):155-68.
6. Camm AJ, Luescher TF, Serruys PW. Choroby serca i naczyń. Podręcznik Europejskiego Towarzystwa Kardiologicznego. Tom I. Poznań: Termedia Wydawnictwo Medyczne; 2006. p. 409-444.
7. Turer AT, Stevens RD, Bain JR. Metabolomic Profiling Reveals Distinct Patterns of Myocardial Substrate Use in Humans with Coronary Artery Disease or Left Ventricular Dysfunction During Surgical Ischemia/Reperfusion. Circulation. 2009;119(13):1736-46.
8. Sabatine MS, Liu E, Morrow DA, et al. Metabolomic Identification of Novel Biomarkers of Myocardial Ischemia. Circulation. 2005;112:3868-75.
9. Chouchani ET, Pell VR, Gaude E, et al. Ischaemic accumulation of succinate controls reperfusion injury through mitochondrial ROS. Nature. 2014; 515(7527): 431–5.
10. Kazmierczak M, Wysocki H, Wykretowicz A, et al. Estimation of hydrogen peroxide plasma levels in patients evaluated for coronary heart disease using dipyridamole infusion followed by SPECT. Coron Artery Dis. 1995;6(1):65-9.
11. Brezinski DA, Nesto RW, Serhan CN. Angioplasty triggers intracoronary leukotrienes and lipoxin A4. Impact of aspirin therapy. Circulation. 1992; 86(1):56-63.
12. Crowie MR, Mosterd A, Wood DA, et al. The epidemiology of heart failure Eur Heart J. 1997;18(2):208-25.
13. Ussher JR, Elmariah S, Gerszten RE, et al. The Emerging Role of Metabolomics in the Diagnosis and Prognosis of Cardiovascular Disease. J Am Coll Cardiol. 2016;68(25):2850-70.
14. Hunter WG, Kelly JP, McGarrah RW, et al. Metabolomic Profiling Identifies Novel Circulating Biomarkers of Mitochondrial Dysfunction Differentially Elevated in Heart Failure with Preserved Versus Reduced Ejection Fraction: Evidence for Shared Metabolic Impairments in Clinical Heart Failure. J Am Heart Assoc. 2016;5(8): e003190.
15. Ahmad T, Kelly JP, McGarrah RW, et al. Prognostic Implications of Long-Chain Acylcarnitines in Heart Failure and Reversibility with Mechanical Circulatory Support. J Am Coll Cardiol. 2016;67(3).
16. Kenneth C, Bedi KC, Snyder NW, et al. Evidence for Intramyocardial Disruption of Lipid Metabolism and Increased Myocardial Ketone Utilization in Advanced Human Heart Failure. Circulation. 2016;133(8):706-16.
17. Norrelund H, Wiggers H, Halbirk M, et al. Abnormalities of whole body protein turnover, muscle metabolism and levels of metabolic hormones in patients with chronic heart failure. J Intern Med. 200;260:11-21.
18. Shane E, Mancini D, Aaronson K, et al. Bone mass, vitamin D deficiency, and hyperparathyroidism in congestive heart failure. Am J Med. 1997;103(3):197-207.
19. Friberg L, Brgfeldt L. Atrial fibrillation prevalence revisited. Intern Med. 2013 Nov;274(5):461-8.
20. Fumaglli S, Said SA, Laroche C, et al. Age-Related Differences in Presentation, Treatment, and Outcome of Patients With Atrial Fibrillation in Europe: The EORP-AF General Pilot Registry. JACC: Clinical Electrophysiology. 2015;1(4):326-34.
21. Mayr M, Yusuf S, Weir G, et al. Combined Metabolomic and Proteomic Analysis of Human Atrial Fibrillation. J Am Coll Cardiol. 2008;51(5):585-94.
22. Ausma J, Coumans WA, Duimel H. Atrial high energy phosphate content and mitochondrial enzyme activity during chronic atrial fibrillation. Cardiovasc Res. 2000;47(4):788-96.
23. Songqing Lai S, Xiumeng Hua X, Gao R, et al. Combinational Biomarkers for Atrial Fibrillation Derived from Atrial Appendage and Plasma Metabolomics Analysis. Nature Sci Rep. 2018;8:16930.
24. Alonso A, Yu B, Sun YV, et al. Serum Metabolomics and Incidence of Atrial Fibrillation (From the Atherosclerosis Risk in Communities [ARIC] Study) Am J Cardiol. 2019 June 15;123(12):1955-61.
25. Elliot P, Andersson B, Arbustini E. Classification of the cardiomyopathies: a position statement from the european society of cardiology working group on myocardial and pericardial diseases. Eur Heart J 2008;29(2):270-6.
26. Lewis GD, Wei R, Liu E, et al. Metabolite profiling of blood from individuals undergoing planned myocardial infarction reveals early markers of myocardial injury. J Clin Invest. 2008;118:3503-12.
27. Jørgenrud B, Jalanko M, Heliö T. The Metabolome in Finnish Carriers of the MYBPC3-Q1061X Mutation for Hypertrophic Cardiomyopathy. PLoS ONE 2015;10(8): e0134184.
28. Pal N, Acharjee A, Ament Z, et al. Metabolic Profiling of Aortic Stenosis and Hypertrophic Cardiomyopathy Identifies Mechanistic Contrasts in Substrate Utilisation. Griffin bioRxiv. October 13, 2019.