Journal of Molecular and Cellular Cardiology 55 (2013) 2–11

Contents lists available at SciVerse ScienceDirect

Journal of Molecular and Cellular Cardiology

journal homepage: www.elsevier.com/locate/yjmcc

Review article

A practical guide to metabolomic profiling as a discovery tool for human heart disease

Lisa C. Heather [a], Xinzhu Wang [b,c], James A. West [b,c], Julian L. Griffin [b,c,*]

[a] Department of Physiology, Anatomy and Genetics, University of Oxford, Parks Road, Oxford, OX1 3PT, UK
[b] The Department of Biochemistry and the Cambridge Systems Biology Centre, Tennis Court Road, Cambridge, CB2 1GA, UK
[c] MRC Human Nutrition Research, Elsie Widdowson Laboratory, 120 Fulbourn Road, Cambridge, CB1 9NL, UK

ARTICLE INFO

Article history:
Received 28 May 2012
Received in revised form 30 November 2012
Accepted 1 December 2012
Available online 8 December 2012

Keywords:
Metabolomics
Metabonomics
Cardiovascular disease
Cardiac metabolism
Functional genomics

ABSTRACT

Metabolomics has refreshed interest in metabolism across biology and medicine, particularly in the areas of functional genomics and biomarker discovery. In this review we will discuss the experimental techniques and challenges involved in metabolomic profiling and how these technologies have been applied to cardiovascular disease. Open profiling and targeted approaches to metabolomics are compared, focusing on high resolution NMR spectroscopy and mass spectrometry, as well as discussing how to analyse the large amounts of data generated using multivariate statistics. Finally, the current literature on metabolomic profiling in human cardiovascular disease is reviewed to illustrate the diversity of approaches, and discuss some of the key metabolites and pathways found to be perturbed in plasma, urine and tissue from patients with these diseases. This includes studies of coronary artery disease, myocardial infarction, and ischemic heart disease. These studies demonstrate the potential of the technology for biomarker discovery and elucidating metabolic mechanisms associated with given pathologies, but also in some cases provide a warning of the pitfalls of poor study design. This article is part of a Special Issue entitled "Focus on Cardiac Metabolism".

Contents

1. Introduction

The heart has a constant demand for adenosine triphosphate (ATP) to power its continual contractile activity, and as such has a high metabolic rate to fulfil this energy demand. Under normal physiological conditions cardiac ATP is predominantly generated by mitochondrial oxidative phosphorylation using fatty acids as a substrate, accounting for 90% of ATP synthesised [1]. A small amount of ATP is generated via anaerobic glycolysis within the cytosol, and is of critical importance when mitochondrial function is limited. Cardiac catabolism can be broadly divided into 3 components—sarcolemmal uptake and cytosolic activation, mitochondrial oxidation and processing in the tricarboxylic acid (TCA) cycle, and finally electron transport and oxygen reduction facilitating ATP synthesis by the electron transport chain and ATP synthase. To maintain ATP production the heart can

Abbreviations: TCA, tricarboxylic acid; GC–MS, gas chromatography mass spectrometry; LC–MS, liquid chromatography mass spectrometry; NMR, nuclear magnetic resonance; PCA, principal components analysis; TMAO, trimethylamine N-oxide.

* Corresponding author at: The Department of Biochemistry and the Cambridge Systems Biology Centre, Tennis Court Road, Cambridge, CB2 1GA, UK. Tel.: +44 1223764922.
E-mail address: jlg40@mole.bio.cam.ac.uk (J.L. Griffin).

0022-2828/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.yjmcc.2012.12.001

metabolise a range of substrates including fatty acids, glucose, ketone bodies, lactate and amino acids [2,3]. The balance between substrates metabolised can vary, both acutely and chronically, according to plasma substrate concentrations, hormones, oxygen availability, workload and disease status [4]. In addition, metabolism of one substrate can suppress the metabolism of alternative substrates, as described in the seminal article by Randle et al. [5].

In many cardiovascular diseases, including myocardial ischaemia, left ventricular hypertrophy and cardiac failure, the heart undergoes a "metabolic shift," amongst other perturbations, modifying substrate selection and utilisation as a consequence of both intrinsic and extrinsic cardiac influences. This metabolic shift has been hypothesised to initially be an adaptive compensatory response but with time to become maladaptive, ultimately contributing to impaired energy production and impaired contractility [4]. In patients with various cardiac pathologies, fatty acid uptake and oxidation have been shown to be decreased and glucose metabolism increased, with these changes correlated with left ventricular end-diastolic diameter and mass [6–8]. In agreement, biopsies from patients with heart failure and aortic stenosis had decreased protein and mRNA expression of fatty acid transporters and key β-oxidation enzymes [9–11]. Glucose uptake and metabolism have also been shown to be increased in patients with dilated cardiomyopathy [6,7]. In addition, cardiac glucose transporter 4 (GLUT4) protein levels have been shown to correlate positively with left ventricular mass index, and negatively with plasma free fatty acids, demonstrating the potential influence of systemic metabolites and cardiac remodelling in influencing the metabolic profile of the heart in human disease [9,12]. Abnormalities in mitochondrial respiration and energetics have also been reported in human heart failure. ADP-stimulated mitochondrial respiration rates in skinned fibres from explanted hearts were decreased compared to healthy control hearts, and activity of complex I of the electron transport chain was also suppressed in patients with dilated cardiomyopathy and coronary artery disease [13,14]. Metabolic abnormalities culminate in impaired myocardial energetics, and the phosphocreatine to ATP (PCr/ATP) ratios was decreased in patients with various cardiac pathologies [15–18], indicating a reduced capacity to buffer the energetics of the cells and transport ATP from the mitochondria to myosin and ATPase ion channels.

2. The role of metabolomics as a metabolic discovery tool for heart disease

It is apparent that large scale perturbations in metabolism and energetics occur in the heart during the development and progression of heart disease. However, many prior studies have been technically limited by the necessity to focus on one pathway or one subsection of metabolism, rather than being able to investigate the metabolome as a whole. Given the highly integrated nature of cardiac metabolism, investigating the complete cardiac metabolic phenotype in one sample would give a far greater understanding of metabolic changes in disease and the identification of unique metabolic signatures specific to certain pathologies. In addition to increasing our understanding of disease progression, metabolomic profiling may also lead to the identification of novel biomarkers, which would aid in the identification and monitoring the progression of disease but would also provide a powerful tool for testing novel pharmaceutical agents and for personalised medicine [19]. The field of metabolomics is a relatively new arrival to the discipline of cardiac metabolism, but the large amounts of information it can generate in relatively short experimental protocols are likely to change the way we approach metabolic research in the future. In this review we focus on a description of the "metabolomic approach" with a particular focus on human cardiovascular disease to demonstrate the benefits such global overviews of metabolism provide. We also refer the reader to the many review articles on metabolomics which include those on cardiovascular disease [20–22] and more general reviews of metabolomics [23,24].

3. The challenges of metabolomics: diversity and complexity

While the aim of metabolomics is to measure all the low molecular weight species in a tissue, biofluid, cell pellet or cell culture media, no analytical tool can completely cover the entire metabolome of even the simplest organism. There are a number of reasons for this. Firstly, unlike transcriptomics and genomics which can utilise polymerase chain reaction, there is no straight forward mechanism to amplify the concentration of metabolites with low abundance. Also, unlike proteomics where the structure of proteins can be reduced to 20 different amino acids which behave relatively similarly in terms of their physicochemical properties, metabolites are a highly diverse range of chemicals existing over a wide mass range and across the extremes of polarity from highly lipophilic to highly hydrophilic. This latter property means that to profile even only the high concentration metabolites in a biofluid or cell requires a range of extraction and chromatographic approaches. However, the biggest challenge is associated with the dynamic range of metabolites, with concentrations ranging from the millimolar for amino acids, sugars and TCA cycle intermediates to picomolar range for signalling molecules, which furthermore may have transient lifetimes.

There is even debate as to the true size of the metabolome. Original estimates were largely based on metabolic pathways that model core metabolism, with initial estimates being modest and in the range of a few hundred metabolites. However, these metabolic charts often ignore the complexity of many of the metabolic classes that make up the cell. For example if we consider acylglycerides as a class of lipid, and hence a subset of the total metabolome of the cell, if one considers there are approximately twenty common fatty acids, this would equate to twenty monoacylglycerides, but approximately 800 diacylglycerides (DAGs) (20*20 1.2 DAGs and an identical number of potential 1.3 DAGs) and 16,000 triacylglycerides! The LipidMaps consortium defines eight broad categories of lipids, which are in turn sub-divided into 83 sub-divisions in their nomenclature for describing lipid species [25,26] and hence the complexity of lipids are apparent. When one considers that sugars can also form polymers as well as modify both lipids and aqueous metabolites it is soon apparent that any estimate of the metabolome must consist of several tens of thousands. Using a combination of laboratory analysis and automated literature mining the human metabolome project has categorized 4299 metabolites in blood serum to date [27]. This demonstrates the real complexity of any description that attempts to describe global metabolism, and a number of on-line databases have been generated to address this complexity, including the human metabolome database (http://www.hmdb.ca/), MassBank (http://www.massbank.jp) and Lipidmaps (http://www.lipidmaps.org/). While there are currently no estimates of the cardiac metabolome a reasonable estimate would be comparable to the serum metabolome, although current technologies are usually limited to the measurement of a few hundred metabolites at most.

4. Extraction procedures for metabolomics: why Folch would recognise the modern metabolomic laboratory

Prior to any analysis, one of the most important steps in a metabolomic study is sample collection. For both biofluids and tissues it is important to prevent degradation of metabolites or the contamination of samples with bacteria which may alter the composition of the mixture. Routinely, metabolism is quenched in tissue by rapidly freezing them, often using liquid nitrogen or dry ice. Blood plasma and serum can be prepared using standard procedures in the clinic, although the best results are obtained if the samples are chilled during this process. The action of bacteria on