IJC Metabolic & Endocrine 8 (2015) 26–30

Contents lists available at ScienceDirect

IJC Metabolic & Endocrine

journal homepage: http://www.journals.elsevier.com/ijc-metabolic-and-endocrine

# Metabolomics in atherosclerosis

Demir Djekic [a], Rachel Nicoll [a], Mehmed Novo [b], Michael Henein [a,*]

[a] Department of Public Health and Clinical Medicine, Umeå University And Heart Centre, Umeå, Sweden
[b] Department of Community Medicine and Rehabilitation, Umeå University, Sweden

ARTICLE INFO

*Article history:*
Received 20 June 2014
Received in revised form 13 November 2014
Accepted 25 November 2014
Available online 3 December 2014

*Keywords:*
Atherosclerotic cardiovascular disease
Metabolomics
Proteomics
Lipidomics
Atherosclerosis

ABSTRACT

It is well established that atherosclerotic cardiovascular disease (ACD) is a leading cause of death in the West. There are several predisposing factors for ACD, which can be divided into two groups: firstly modifiable risk factors, including hypertension, dyslipidaemia, type 2 diabetes mellitus, obesity, smoking and a sedentary lifestyle and secondly the unmodifiable risk factors such as age, gender and heredity. Since single biomarkers are unable to provide sufficient information about the biochemical pathways responsible for the disease, there is a need for a holistic approach technology, e.g., metabolomics, that provide sufficiently detailed information about the metabolic status and assay results will be able to guide food, drug and lifestyle optimisation. Rather than investigating a single pathway, metabolomics deal with the integrated identification of biological and pathological molecular pathways. Mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy are the two most commonly used techniques for metabolite profiling. This detailed review concluded that metabolomics investigations seem to have great potential in identifying small groups of disturbed metabolites, which if put together should draw various metabolic routs that lead to the common track pathophysiology. The current evidence in using metabolomics in atherosclerotic cardiovascular disease is also limited, and more well-designed studies remain to be established, which might significantly improve the comprehension of atherosclerosis pathophysiology and consequently management.


## 1. Introduction

It is well established that atherosclerotic cardiovascular disease (ACD) is a leading cause of death in the West [1]. It is a chronic inflammatory disorder [2] that begins in early childhood with fatty streak formation in the coronary arteries [3] and remains silent until late adulthood, when lipids start to accumulate in the intima forming atherosclerotic plaques [4]. There are several predisposing factors for ACD, which can be divided into two groups: first, modifiable risk factors, including hypertension, dyslipidaemia, type 2 diabetes mellitus, obesity, smoking and a sedentary lifestyle and, second, the unmodifiable risk factors such as age, gender and heredity [5]. Ageing is the predominant risk factor for ACD, and it renders many of the modifiable risk factors more prevalent and more severe [6]. Thus, the aetiology of atheroma formation is multifactorial with a synergistic effect of many risk factors. At present, cardiologists remain using conventional risk factors, derived from the Framingham studies [7]. Although helpful, those risk factors do not detect all instances of CAD, and indeed a significant percentage of patients with myocardial infarction has no conventional risk factors [8]. Other markers such as coronary calcification have been found to improve risk stratification but still fall a long way short of 100% prediction [8]. Conventional coronary angiography is invasive, time consuming and expensive and subjects the patient to radiation, while computed tomography coronary angiography (CTCA) also suffers from many of the same limitations.

On the other hand, single disease biomarkers are always desired to identify risk factors or people affected by a disease to evaluate progress or to monitor an intervention in treating the disease. This principle has been shown useful for, e.g., bacterial infections, where a specific molecule or groups of molecules are characteristic of the disease state and largely distinctive within the matrix being sampled. The commonly investigated biomarker in ACD is cholesterol. However, serum cholesterol levels may fail to identify the exact pathway to their abnormal levels since sources of increased cholesterol levels are not only food but also endogenous biosynthesis or slow conversion to bile acids [9]. Since single biomarkers are unable to provide sufficient information about the biochemical pathways responsible for the disease, there is a need for a holistic approach technology, e.g., metabolomics, that provide sufficiently detailed information about the metabolic status and assay results will be able to guide food, drug and lifestyle optimisation [9].

The metabolome refers to the complete set of small molecule (low molecular weight (<1500 Da)) metabolites in a cell, tissue, organ or organism [10], while metabolomics is the comprehensive analysis and quantification of these small molecules based on biofluids and tissue

* Corresponding author.
*E-mail address:* Michael.Henein@medicin.umu.se (M. Henein).

http://dx.doi.org/10.1016/j.ijcme.2014.11.004

analysis [11,12]. Rather than investigating a single pathway, metabolomics deal with the integrated identification of biological and pathological molecular pathways [13].

Roger William together with colleagues were the first pioneers who introduced the concept, in late 1940s, that individuals might have a metabolomics profile, which could be reflected in the 'structure' of the biological fluids. By utilising paper chromatogram, William examined taste threshold and the excretion profiles from alcoholics and schizophrenics. He linked each of these disorder with a specific metabolic profile [14]. In 1960s and 1970s, gas chromatography and liquid chromatography further advanced the technique, which made them more available [15]. The term metabolic profile was first introduced by Hornings et al. in early 1970s [16]. They suggested that metabolic profiles may be valuable for characterising both normal and disease state. The '-omics' approach includes genomics, transcriptomics, proteomics and the rapidly emerging metabolomics [17].

Mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy are the two most commonly used techniques for metabolite profiling [18]. Each of these techniques has important strengths and weaknesses. For example, MS is innately more sensitive than NMR but requires a prior separation of metabolites using chromatography (liquid or gas chromatography) or capillary electrophoresis (CE) [19]. Further, the ionisation or ion suppression effect could impair the analytic quantification. The MS can, with standard techniques, detect smaller metabolites than NMR (picomolars for MS and micromolars for NMR), and those metabolites detected with NMR need to contain a hydrogen atom. By using NMR sample, recovery is non-destructive, and the sample is analysed in only one measurement while for MS only a small amount of sample is used that eventually will be destructed [20].

Both techniques can be used to characterise metabolic data in a targeted or non-targeted aspect. For the targeted approach, the investigator focuses on a limited set of metabolites of known identity. Untargeted approach deals with the investigation of as many peaks as possible, with unknown underlying identities of the species. Therefore, untargeted analyses are considered more sensitive and more likely to discover new biomarkers, while target analyses are used in biomarker validation [21]. Because of data complexity, NMR can assign definitive metabolite identities to only a subset of peaks arising from the sample. Although MS can generate sometimes thousands of peak metabolites from the biofluids, chromatographic extraction time and mass to change ratio are often insufficient to confidently assign peak intensities [21]. The organisation of the human metabolome database (HMDB) is a comprehensive online database that gathers small molecule metabolites found within the human body. The HMDB second version, produced in 2009, was able to identify 6500 metabolites [22] and the latest version available from 2013 has significantly expanded to identify more than 40.000 metabolites [23].

During the last decade, there has been growing interest in the application of metabolomics for early disease prevention and to potentiate drug development and therapy monitoring [8,9]. In the setting of coronary atherosclerosis, metabolomics has been shown to identify a number of disordered biomarkers, some of which may be susceptible to modification. The development of a set of metabolites which could aid prediction of ACD would certainly be welcomed. Furthermore, it is possible that urine or salivary samples may be used for metabolic analysis, which even avoids the need for phlebotomy. In this review, we will discuss the latest metabolomic approaches within the field of ACD and its related risk factors.

## 2. Atherosclerosis

### 2.1. Animal studies

Lyso-phosphatidylcholine (LPC) is a class of phospholipids that are intermediates in the metabolism of lipids. LPC is generated by an enzyme found on oxidised LDL called lipoprotein-associated phospholipase A2 from phosphotidylcholine [24]. Increased levels of this enzyme have been associated with higher risk of developing ACD [25]. LPC is involved with early pro-inflammatory events occurring in the initial state of a plaque formation by increasing adhesion of monocytes on endothelial cells, as well as activating inflammatory genes (NF-KB) during fatty streak development. This leads to the activation of apoptosis, necrosis and formation of cholesterol crystals which causes plaque vulnerability [26]. Clish et al. [27] found that using highly performing liquid chromatography–mass spectrometry (HPLC-MS), lyso-PC was elevated in the liver of the apolipoprotein E3 (Apo E3) Leiden transgenic mouse (susceptible to development of ACD) in contrast to controls.

In another study by Kleemann et al. [28], ApoE Leiden mice were fed a low *vs.* high cholesterol diet. The amount of dietary cholesterol positively correlated with development of atherosclerosis. With increasing dietary cholesterol intake, the liver switched from a mainly resilient to a predominantly inflammatory state, which is associated with early lesion formation. The high cholesterol evoked changes involving specific transcriptional master regulators, some of which are established, others newly identified, several of these regulators control both lipid metabolism and inflammation and thereby link the two processes. The liver and plasma were analysed with HPLC/MC identified disturbance in di-and triglycerides, phosphatidylcholines, lysophosphatidylcholines and cholesterol esters.

The effect of different diets on atheroma formation has been also studied. Martin et al. investigated the effect of several diets fed to hyperlipidaemic hamsters and observed that aortic cholesteryl ester, assessed by NMR spectroscopy, was an early accumulator in atherogenic plaques. The lowest atherogenicity was obtained with the plant-oil cheese diet, followed by the dairy fat cheese diet, while the greatest atherogenicity was observed with the butter diet. Aortic cholesteryl ester was positively correlated with very low density lipoprotein (VLDL), cholesterol and *N*-acetylglycoproteins and negatively correlated with trimethylamine-*N*-oxide (TMAO) and albumin lysyl [29].

Likewise, Jove et al. observed that a high-fat diet caused an increase in ceramide and docosahexaenoic acid (DHA) in plasma and a tissue sample from the aorta. Free cholesterol in the aorta was positively correlated with taurocholic acid, suggesting that it could be a biomarker for early atherogenesis [30]. Furthermore, a high-fat cholesterol choate (HFCC) diet has been shown to alter plasma and urinary metabolism in LDL-receptor-deficient mice using H-NMR spectroscopy. The HFCC diet caused a significant perturbation in choline metabolism, notably the choline oxidation pathway a significant reduction in the urinary excretion of taurine, betaine and dimethylglycine [31].

In addition to these studies, recent ones showed that not only diet and liver have a role in the development of atherosclerosis but also gut floras have demonstrated great importance. It has been suggested that microbiome, i.e., the collective genomes of the microorganisms that reside in the gut, can increase cardiovascular risk either via metabolism of L-carnitine [32] or phosphatidylcholine (Wang at al 2011). Mice were given either a control diet or a diet rich in choline. The gut microflora was suppressed in half of the mice given broad-spectrum antibiotics [33]. Upon analyzing the plasma, TMAO was suppressed to a non-detectable level. The development of the aortic root lesion was increased 3-fold in mice not treated with antibiotics, and hence with preserved gut microflora, and in those fed a choline-enhanced diet. Thus, Wang et al. supposed that dietary supplements of choline, TMAO and betaine (which are all metabolites of phosphatidylcholine) could enhance the development of atherosclerosis. Furthermore, it was proposed that these metabolites are involved in the upregulation of scavenger receptors on macrophages hence the formation of foamy cells.

In another study, Stöhr et al. [34] also linked atherosclerosis to the metabolism of carnitine. By using a targeted metabolomic approach, the group studied the plasma of genetically modified mice susceptible to atherosclerosis. Interestingly, they observed a decrease in the blood concentration of free carnitine, acetylcarnitine and glutarylcarnitine/3-hydroxy-hexanoylcarnitine.