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Metabolomic profiles of lipid metabolism, arterial stiffness and hemodynamics in male coronary artery disease patients

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ABSTRACT

Background/objectives: Metabolomic profiling allows to take a detailed look at lipid metabolism and to study the levels and roles of its numerous intermediates and products in the pathogenesis of cardiovascular disease (CVD). This study aimed to investigate the relationship between the metabolic profiles of lipid metabolism, arterial stiffness and hemodynamics in patients with coronary artery disease (CAD) and for healthy controls.
Methods: Serum levels of 186 metabolites were determined with the AbsoluteIDQ™ p180 kit (BIOCRATES Life Sciences AG, Innsbruck, Austria). The technique of applanation tonometry was used for non-invasive pulse wave analysis and carotid-femoral pulse wave velocity (cf-PWV) assessments. Principal component analysis (PCA) was carried out in order to reduce the large number of correlated metabolites to fewer uncorrelated factors.
Results: Elevated levels of C16:1, C18:1, C3-DC(C4-OH), PC aa C40:6, Met-SO/Met, and reduced levels of lysoPC a C18:2 were observed in the CAD group compared to the healthy controls. The cf-PWV showed positive correlations with C14, C16:1, (C2 + C3) / C0, C2 / C0 and the CPT-1 ratio for the CAD group. Moreover, PCA-derived factor 3 (medium- and long-chain acylcarnitines) proved to be an independent determinant of cf-PWV for these patients.
Conclusions: We demonstrated an independent association between the serum medium- and long-chain acylcarnitine profile and aortic stiffness for the CAD patients. In addition to the lipid-related classical CVD risk markers, the intermediates of lipid metabolism may serve as novel indicators for altered vascular function.

1. Introduction

The levels of the lipid metabolism-related classical biomarkers (i.e. total cholesterol, LDL cholesterol, HDL cholesterol and triglycerides) have been estimated for decades to assess the risk for cardiovascular disease (CVD). However, recent analytical developments (e.g. metabolomics) may enable to extend the current biochemical signature for both CVD prognosis and risk stratification.

Metabolomics is an emerging discipline for profiling low-molecular-weight metabolites in health and disease. Assessment of the metabolome provides insight into the actual metabolic and physiological state of a specific cell, organ, or organism. This information allows to gain a more profound understanding of the pathogenic mechanisms that lead to the morbid state. Furthermore, metabolomic profiling could have particular clinical relevance in early detection, diagnosis and prognosis of both acute and chronic diseases. It is therefore essential to unlock the true potential of this discipline through linking metabolomic data to known and validated clinically relevant biochemical and functional biomarkers.

Metabolomic profiling allows us to take a more detailed look at lipid metabolism and to study the levels and roles of its numerous intermediates and products in the pathogenesis of CVD. In fact, metabolomic profiling of CVD has been in the focus of some recent large-scale studies. Using a targeted mass spectrometry-based approach, Shah and colleagues determined metabolic signatures composed of dicarboxylacylcarnitines, medium-chain acylcarnitines, and fatty acids that were independently predictive of future CV events in patients at risk for coronary artery disease (CAD) [1]. A small study in very old subjects with a high rate of previous CVD also suggested that medium- and long-chain acylcarnitines could be independently associated with the subsequent occurrence of CV events [2]. In addition, certain lysophosphatidylcholines (lysoPC) – such as lysoPC 18:1 and 18:2 – were found to be the risk factors associated with CAD in a recent large-scale prospective cohort study conducted by Ganna et al. [3]. The results of these studies clearly indicate that the signature of metabolic profiles may be associated with the increased risk of CAD and adverse CV outcomes. However, considering the still emerging role of metabolomics in CV research, all novel associations between certain metabolites and CVD need to be repeatedly inspected by different groups using different methodological

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approaches. In this way, it would be possible to gather sufficient data to establish the mechanisms by which these metabolites may participate in the development of CVD.

Arterial stiffness is characterized by the reduced capability of a blood vessel to dilate and constrict in response to changes in blood pressure. It is an independent predictor of all-cause and CV mortality in a range of different diseases and in the general population [4–7]. Identification of metabolic signatures of arterial stiffness in CAD patients might help better understand the impact of the different low-molecular-weight metabolites – especially the ones related to changes in lipid metabolism – on the pathogenesis of CVD.

The main objective of this study was to investigate the relationship between the metabolic profiles of lipid metabolism, arterial stiffness and hemodynamics in patients with CAD and in clinically healthy controls.

2. Subjects and methods

2.1. Study population

The present study included 52 male patients with angiographically confirmed CAD, who were prospectively recruited from the Department of Cardiology, University of Tartu, Estonia. The exclusion criteria were any comorbid acute or chronic inflammatory disease, diabetes mellitus, myocardial infarction, cerebrovascular events or revascularization operation during the preceding 6 months, unstable angina, cardiac arrhythmias, clinically significant heart failure or valvular disease, reduced kidney function (eGFR < 60 ml/min/1.73 m^2), presence of cancer or endocrine pathology.

A total of 40 age- and gender-matched clinically healthy controls from the same geographic region were identified through local family physicians. In this group the exclusion criteria were any comorbid acute or chronic inflammatory disease, CAD, cerebral or peripheral atherosclerotic disease, diabetes mellitus, cardiac arrhythmias, clinically significant heart failure or valvular disease, hypertension, reduced kidney function (eGFR < 60 ml/min/1.73 m^2), presence of cancer, infectious disease or endocrine pathology, or regular use of any medication.

2.2. Study protocol

Lifestyle factors and medical history were obtained using an interview and a self-completed questionnaire. Venous blood samples were drawn from all subjects between 8 am and 11 am after an overnight fast and abstinence from tobacco, alcohol, tea and coffee. Height and weight were measured and body mass index (BMI) was calculated. The subjects were studied after 10 min of rest in a supine position in a quiet, temperature-controlled room. Brachial blood pressure and carotid-femoral pulse wave velocity (cf-PWV) were assessed and pulse wave analysis was made. All hemodynamic measurements were taken in duplicate and averaged. Each subject gave his written informed consent, and the study protocol was approved by the Ethics Committee of the University of Tartu.

2.3. Arterial stiffness and central hemodynamics measurements

Central aortic waveforms and other hemodynamic parameters were evaluated non-invasively with the commercially available SphygmoCor apparatus (AtCor Medical, Sydney, Australia) [8]. After 15 sequential high-quality radial waveforms were recorded at the patient's left wrist using arterial applanation tonometry, a validated generalized transfer function for calculation of the central aortic pressure waveform was used [9,10]. Augmentation index (%) was defined as the difference between the second and first systolic peaks of the central arterial waveform, expressed as a percentage of the central pulse pressure [11]. Carotid-femoral pulse wave velocity (cf-PWV), a measure of aortic stiffness, was calculated from the pulse transit time using ECG-gated carotid and femoral artery waveform recordings over a known distance [8]. The distance from the suprasternal notch over the umbilicus to the femoral artery minus carotid arterial length was used for calculation of cf-PWV.

2.4. Biochemical analysis

Peripheral venous blood samples were collected in serum separator tubes (BD SST™ II Advance) and plain tubes (Plain BD Vacutainer® Tubes) for clinical biochemistry analysis and for metabolomic analysis, respectively. Thereafter, all samples were centrifuged and the supernatant was pipetted into Eppendorf tubes. The tubes were stored at −70 °C until assayed. Serum myeloperoxidase (MPO), oxidized low-density lipoprotein (oxLDL) and adiponectin levels were measured using an enzyme-linked immunosorbent assay (ELISA) (Myeloperoxidase enzyme immunoassay test kit, BIOCHECK, Inc. Foster City, CA; Merdcodia Oxidized LDL ELISA, Uppsala, Sweden; Human Total Adiponectin/Acrp30 immunoassay, R&D Systems Europe, Abingdon, UK, respectively). The serum levels of interleukin-6 (IL-6) were determined using The Evidence Investigator (Metabolic Syndrome Array-1, Randox Laboratories, Crumlin, UK). The plasma concentrations of total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, glucose, white blood cell (WBC) count, high-sensitivity C-reactive protein (hs-CRP) and eGFR were measured in a local clinical laboratory with automated analyzers using standard laboratory methods.

2.5. Targeted metabolite quantification

The serum levels of metabolites were determined with the AbsoluteIDQ™ p180 kit (BIOCRATES Life Sciences AG, Innsbruck, Austria) using the flow injection analysis tandem mass spectrometry (FIA–MS/MS) as well as liquid chromatography ((LC)–MS/MS) techniques. All measurements were performed as described in the manufacturer's manual UM-P180. The AbsoluteIDQ™ p180 kit allows simultaneous quantification of 186 metabolites (acylcarnitines (Cx:y), amino acids and biogenic amines, hexose, sphingolipids (SMx:y or SM (OH)x:y), glycerophospholipids (lysophosphatidylcholines (lyso PCx:y) and phosphatidylcholines (PCaa x:y and PC ae x:y)) from 10 μl of serum. Lipid side-chain composition is abbreviated as Cx:y, where x denotes the number of carbons in the side chain and y denotes the number of double bonds. Glycerophospholipids are differentiated further according to the presence of ester (a) and ether (e) bonds in the glycerol moiety. Double letters (aa = diacyl, ae = acyl–alkyl) indicate that two glycerol positions are bound to a fatty acid residue, while a single letter (a = acyl or e = alkyl) indicates a bond with only one fatty acid residue. Identification and quantification of the metabolites was achieved using multiple reaction monitoring (MRM) along with internal standards. Calculation of metabolite concentrations was automatically performed by the MetIDQ™ software (BIOCRATES Life Sciences AG). The concentrations of all metabolites were calculated in μM. Metabolites below the limit of detection (n = 35) determined experimentally by BIOCRATES were excluded from further analysis. The assay procedures of the AbsoluteIDQ™ p180 kit have been described in detail previously [12].

2.6. Statistical analysis

The normality of the distribution of continuous variables was assessed using the Shapiro–Wilk's test. The metabolite serum concentrations were logarithmically transformed as they were positively skewed. Analysis of covariance was employed to adjust metabolite levels for BMI and statin use. To account for multiple testing the Benjamini–Hochberg procedure was used to control false discovery rate (FDR) at the level of 0.05 [13]. Differences between the two groups were evaluated by two-tailed Student's t-test and the Mann–Whitney U test, where appropriate. Pearson correlation and Spearman rank