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# Interventional left atrial appendage closure may affect metabolism of essential amino acids and bioenergetic efficacy

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ABSTRACT

*Background:* Interventional closure of left atrial appendage (LAAC) represents an alternative for stroke prevention in patients with non-valvular atrial fibrillation. Whether LAAC may affect metabolomic pathways has not been investigated yet. This study evaluates the impact of LAAC on the metabolism of essential amino acids, kynurenine and creatinine.
*Methods:* Peripheral blood samples of prospectively enrolled patients undergoing successful LAAC were taken before (T0) and 6 months after (T1, mid-term follow-up). Targeted metabolomic profiling was performed using electrospray ionization liquid chromatography–mass spectrometry (ESI-LC-MS/MS) and MS/MS measurements focusing on metabolism of essential amino acids.
*Results:* 44 patients with non-valvular AF (mean CHA2DS2-VASc score 4, mean HAS-BLED score 4) were enrolled. Changes in metabolites of essential amino acids, myocardial contraction and bioenergetic efficacy, such as phenylalanine (percentage change 8.2%, $p = 0.006$), tryptophan (percentage change 20.3%, $p = 0.0006$), tyrosine (percentage change 20.2%, $p = 0.0001$), creatinine (percentage change 7.2%, $p > 0.05$) and kynurenine (percentage change 8.3%, $p = 0.0239$) were found at mid-term follow-up.
*Conclusions:* LAAC may affect the metabolism of essential amino acids and bioenergetic efficacy.
ClinicalTrials.gov Identifier: NCT02985463



## 1. Introduction

With steadily rising number of affected patients, atrial fibrillation is the most frequent arrhythmia in the Western World. Complications may be life-threatening including stroke, worsening of heart failure and sudden death. [1] The main hallmarks of the management of non-valvular AF are rhythm control, heart rate control and stroke prevention depending on the $CHA_2DS_2$-VASc score. The left atrial appendage (LAA) plays a pivotal role in stroke development. In the last years the interventional exclusion of the LAA has risen to an established alternatively method to prevent strokes in patients with non-valvular AF. [2] Left atrial appendage closure (LAAC) is recommended in patients with a contraindication for oral anticoagulation (OAC) or in patients who suffered from a stroke despite OAC treatment. [1,2]

However, LAAC might affect cardiac pathophysiology. Cardiac biomarkers such as Atrial natriuretic peptide (ANP) and B-type natriuretic Peptide (BNP) may decrease after LAAC. [3,4] Several studies demonstrated that cardiac interventions may influence cardiac remodeling. [5–7] Other studies showed that cardiac surgery influences gene and protein expression. [6,8]

Metabolomics have become of high interest in the last decades and are implemented in modern cardiovascular research. [9–11] It was described recently, that cardiac resynchronization therapy (CRT) might alter metabolomic profiles. [7,12] The heart represents one of the most metabolically demanding organs, and therefore associations between metabolomics and cardiovascular diseases rise certain hope for novel diagnostic and prognostic tools. [9,13] In a prospective study including 212 patients with acute decompensated heart failure, Wang et al. could demonstrate that a metabolic score consisting of butyrylcarnitine, dimethylarginine/arginine ratio, spermidine, and total essential amino acids was associated with a worse outcome and a higher incidence for AF. [14] Furthermore, a sub-analysis of the Framingham study population, showed no association of tested metabolites including amino acids, organic acids and lipids in patients with new onset of

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atrial fibrillation. [15] This shows the controversial data existing in metabolomic profiling of patients with AF.

However, we recently demonstrated significant changes of metabolites of nutrient processing, namely glycolysis, tricarboxylic acid and protein metabolism, potentially mirroring remodeling processes of the left atrium and ventricle ongoing after LAAC. [16,18] Based on our experience in this field, this study therefore evaluates whether LAAC might affect metabolism of essential amino acids, kynurenine and creatinine, potentially reflecting alterations of bioenergetics efficacy and myocardial contraction.

## 2. Methods

### 2.1. Study population

The "Left Atrial Appendage Occlusion and Biomarker Evaluation" (LABEL) study (ClinicalTrials.gov Identifier: NCT02985463) is a single-center, prospective, hypothesis generating, observational non-randomized study including patients eligible for percutaneous LAAC according to the guidelines of the European Society of Cardiology. [17] As previously being published by our working group [16,18], all patients presented with non-valvular AF, a CHA2DS2-Vasc score ≥ 2, a HAS-Bled score ≥ 3, and a contraindication for the therapy with oral anticoagulants, such as major or recurring bleeding. Exclusion criteria were age < 18 years, congestive heart failure classified as NYHA IV, catheter ablation of AF within 30 days prior to planned intervention, myocardial infarction within the last 3 months, atrial septum defect or implanted ASD occluder, mechanical heart valves, status after heart transplant, symptomatic carotid artery stenosis, transient ischemic attack or stroke within 3 months, existing or planned pregnancy, or acute infection or detection of intracardiac thrombus at the day of planned implantation. The study was carried out according to the declaration of Helsinki and was approved by the medical ethics committee II of the Faculty of Medicine Mannheim, University of Heidelberg, Germany. Written informed consent was obtained from all participants or their respective legal representative.

### 2.2. Characterization of the study population and changes over the mid-term follow-up

At T1 and T2, the following characteristics were thoroughly assessed in-hospital and in collaboration with general practitioners on the basis of clinical judgment: relevant changes of body weight or dietary habits (i.e., nutritional status), clinical signs of heart failure (e.g., dyspnea, edema), renal failure (e.g., fatigue, pruritus, edema), (pre)diabetes mellitus (e.g., fatigue, polydipsia, polyuria) and local or systemic inflammation. In addition, BMI, cholesterol, low- and high-density lipoproteins, triglycerides, LV function, NT-proBNP, creatinine level and glomerular filtration rate calculated by the Modification of Diet in Renal Disease (MDRD) formula, average blood glucose level, C reactive protein (CRP), and lactate dehydrogenase (LDH) were measured and assessed at baseline. These parameters were re-assessed occasionally during follow-up on the basis of clinical judgment according to the patients' individual history or clinical deterioration.

### 2.3. Implantation of LAA occlusion devices and follow-up

Percutaneous closure of the LAA was performed using either the Watchman (Boston Scientific, Marlbrough, MA, USA) or Amplatzer Amulet (St. Jude Medical, St. Paul, MN, USA) device. Successful closure of LAA was confirmed by transesophageal echocardiography (TEE) during index procedure, as well as at mid-term follow-up by TEE and cardiac computed tomography angiography (CCTA). Incomplete closure of the LAA was an exclusion criterion and therefore solely patients with complete closure of the LAA were included. All relevant clinical data regarding prior medical history, medication (such as beta blocker, ACE inhibitor, aldosterone antagonists, diuretics), actual symptoms and laboratory data were documented in detail both at baseline as well as follow-up.

### 2.4. Sample preparation

Blood samples were taken from each patient by venous puncture within 24 h prior to cardiac intervention (T0) and at least 6 months later (T1, mid-term follow-up). Plasma was generated by centrifugation at 2500 x g for 10 min at 20 °C. The aliquoted samples were snap frozen in liquid nitrogen and stored at −80 °C until analysis. Processing was performed within two hours after blood extraction.

### 2.5. Metabolite analysis

A targeted metabolomics approach based on electrospray ionization liquid chromatography–mass spectrometry (ESI-LC-MS/MS) and MS/MS measurements was performed using the AbsoluteIDQ™ p180 Kit (BIOCRATES Life Sciences AG, Innsbruck, Austria). The assay allows simultaneous quantification of in total 188 metabolites out of 10 μl plasma samples, including tryptophan, phenylalanine, tyrosine, leucin, isoleucine, valine, threonine, creatinine and kynurenine. Analyses were carried out on a QTRAP 4000 System (Sciex Deutschland GmbH, Darmstadt, Germany) and a Thermo TSQ (ThermoFisher Scientific, Waltham, USA). All samples were prepared and measured simultaneously, being randomly allotted to two different assay plates. For the evaluation of metabolite concentrations, internal standards served as a reference. Metabolites were defined as MSI level I metabolites. BIOCRATES MetIDQ™ software was used for the processing and technical validation of the metabolite data.

### 2.6. Statistical analysis

To exclude metabolites of which concentration values were below LOD, a general cleaning of the data set based on an 80% rule was performed. Remaining values below LOD in the data set were then imputed applying a logspline imputation method. The resulting data set was log2 transformed. [19,20] Principal Component Analysis (PCA), Partial Least Squares Discrimination Analysis (PLS-DA) and Hierarchical Cluster Analysis (HCA) were used as supervised and unsupervised multivariate approaches [21]. To compare significant differences, data were subjected to a student's *t*-test or repeated measures ANOVA (rANOVA). The rANOVA was used for the correlated patient samples to detect overall changes between the means over the two time points (independent variable) including the different clinical parameters (dependent variables). All statistical tests were performed on the log-transformed data, that follow a normal distribution. To control the False-Discovery-Rate (FDR) during multiple comparisons, an adjusted *p*-value (Benjamini-Hochberg correction) was additionally calculated. [22] Percentage change: [(Mean value T1 / Mean value T0) − 1] × 100; Fischer ratio = BCAA / AAA = (valine + leucine + isoleucine) / (tyrosine + phenylalanine).

A regression analysis based on a linear mixed effect model was applied for the evaluation of dependency of significant metabolite change on clinical factors which had shown absolute changes in metabolite concentration between T0 and T1 (gender, age, diabetes mellitus, body mass index, left ventricular ejection fraction, creatinine, pro-B natriuretic peptide). For creatinine, age and pro-BNP cutoffs were the estimated medians to dichotomize the data. Data are presented as medians with interquartile ranges (IQR) (i.e. 25.-75. percentiles). Statistical analysis was performed using R-Studio. [23]

## 3. Results

### 3.1. Study population

44 patients with a median age of 78 years were included and successfully underwent LAAC. At the time of enrolment 77% of patients had experienced a major bleeding and showed a median HAS-BLED score of 4 (interquartile range [IQR] 3–4.3). Nearly a quarter of the study cohort had experienced a stroke or TIA before enrolment. Median CHA2DS2-VASc score was 4 (IQR 3–5). More than half of the patients had a known history of coronary artery disease (CAD). At mid-term follow up nearly 16% of the cohort suffered from overt bleeding (BARC-Score 2 and 3a). Most of the patients enrolled in the study showed a normal left ventricular function (LV-F). Altogether, the collective of this study had an increased cardiovascular risk profile with high proportion of hypercholesterinemia (50%), arterial hypertension (95%) and diabetes mellitus (37%). All demographic and clinical characteristics of the patients are outlined in Table 1.

Based on a thorough clinical assessment, there were no relevant changes in the overall clinical status over mid-term follow-up, as reflected by a stable course regarding nutritional and smoking status, pharmacotherapy, heart failure symptoms, anemia, renal function, lipid status, inflammation, and left ventricular function (each $p > 0.05$ between T1 and T2; data not shown). New onset of diabetes was not observed at mid-term follow-up.

### 3.2. Metabolic alterations after LAAC

Supplemental Fig. 1 shows an overview of analyzed metabolites prior to intervention (T0) and after 6 months (median 182 days (IQR 175–182 days) (T1). Mean percentage changes before and after LAAC (plasma metabolite concentrations prior intervention [T0]/plasma metabolite concentrations after intervention [T1]) of the metabolic profile are demonstrated in Supplemental Fig. 2. Analyses of patients' plasma showed an increase of valine, leucin and isoleucine as well as an increase in creatinine levels. Significant changes could be determined in phenylalanine, tryptophan, tyrosine and kynurenine plasma concentrations. Table 2 shows the alterations of the metabolic profile with indication of the percentage changes and *p*-values. Furthermore, metabolite ratios of tyrosine/phenylalanine and kynurenine/tryptophan are given in Table 2 showing that the metabolites ratio might increase or drop even though the percentage changes of the single metabolites might

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