Fecal short chain fatty acids role in atrial fibrillation paroxysm pathogenesis through coronary artery disease patients

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Abstract: gut microbiota composition and its metabolites is an essential part of human health. Short chain fatty acids (SCFA) are known gut microbiota metabolites. Lack of them is common for dyslipidemia and inflammatory changes. But their role in atrial fibrillation (AF) and coronary artery disease (CAD) pathogenesis is still uninvestigated. The aim: to estimate the fecal short chain fatty acids changes in patients with atrial fibrillation paroxysm and coronary artery disease and found their connections with known cardiometabolic risk factors. Materials and methods: 300 patients were investigated. We divided them into 3 groups: I group – 149 CAD patients without rhythm disorders, II group – 124 patients with CAD and AF paroxysm and control group (CG) – 27 patients without CAD and arrhythmias. Fecal SCFA was checked by gas chromatography with mass electron detection. Results: Fecal SCFA changes in patients with AF paroxysm and CAD were found in our investigation. Isocaproic and isobutyric fecal acids appears in CAD and AF patients' samples in comparison with control group. In the patients with AF and CAD significant increasing of valeric (1128,43%) and decreasing butyric (78,75%), isovaleric (56,29%), caprylic (99,21%) acids, medium chain fatty acids (95,54%) and unsaturated fatty acids (38,76%) levels was revealed in comparison with CAD patients without arrhythmias (P<0,05). The largest amount of correlations was between total amount of SCFA, medium chain fatty acids (total amount = 7), butyric acid (total number = 6) and cardiometabolic risk factors (P<0,05). The acceptable role of total amount of short chain fatty acids (AUC = 0.7907) and butyric acid (AUC = 0.7127) in AF paroxysm occurrence in CAD patients was proven by ROC-analysis. Conclusions: SCFA-synthesis violations were revealed in patients with atrial fibrillation paroxysm and coronary artery disease. To propose the new ways of gut microbiota and cardiometabolic risk factors correction will be interesting for future investigations.

Keywords: Coronary Artery Disease, Atrial Fibrillation, Fatty Acids, Cardiometabolic Risk Factors, Patients.

Introduction
Atrial fibrillation (AF) is the most common arrhythmia in the world. By the latest data more than one third European population over 55 suffers from AF. In projection increase of AF will be growth each year. One of the know AF risk factor is coronary artery disease (CAD) (Hindricks G. et al., 2020). CAD is the most common cardiovascular pathology in the world. Its spreading is also increasing (Knuuti J. et all., 2020). One of explanation of such demographic picture is that CAD and AF have a lot of identical risk factors: dyslipidemia, smoking, obesity, diabetes, obstructive sleep apnea, inflammatory diseases, sedentary way of life, etc. (Hindricks G. et all., 2020; Knuuti J. et all., 2020). Moreover,
CAD and AF presence worsening clinical picture and prognosis of each other, increase risks of cardiovascular events (Michniewicz E. et al., 2018).

The majority of CAD and AF risk factors are pathogenetically linked with gut microbiota condition and its metabolites. By the literature data, inflammatory and metabolic disorders are strongly linked with dysbiosis presence. Gut microbiota violations can act at host organism by its metabolites, which rises in blood flow due to increasing intestinal barrier permeability and their production lesions. Gut microbiota metabolites are trimethylamine (TMA), trimethylamine-N-oxide (TMAO), lipopolysaccharide, bile acids and short chain fatty acids (SCFA) (Malesza IJ et al., 2021; Scheithauer TPM et al., 2020). TMA, TMAO are well known gut microbiota metabolites. Their role in AF paroxysm and CAD pathogenesis is widely discussed nowadays (Gatarek P et al., 2021). Lipopolysaccharide (endotoxin) is also presented as AF paroxysm risk factor (Zhang Y et al., 2022). Elevated levels of circulated bile acids are a well-known AF risk factor (Michelle SW et al., 2019).

SCFA is a crucial gut microbiota metabolite in regulating host immune homeostasis. SCFA are synthetized in human intestine from dietary fibers through fermentation by microorganisms. Deficiency of SCFA content leads for cardiovascular and metabolic disorders by the latest evidence. Some laboratory findings suggested the importance of SCFA role in AF paroxysm development (Lizogub V.G. et al., 2019; Ling Z. et al., 2022), but any clinical investigations. Moreover, it is no evidence about SCFA role in AF paroxysm in CAD patients.

**Aim**

The aim to estimate the fecal short chain fatty acids changes in patients with atrial fibrillation paroxysm and coronary artery disease and found their connections with known cardiometabolic risk factors.

**Materials and methods**

300 patients were investigated. We divided them into 3 groups: I group – 149 CAD patients without rhythm disorders, II group – 124 patients with CAD and AF paroxysm and control group (CG) – 27 patients without CAD and arrhythmias. All diagnosis was established according current European Society of Cardiology guidelines (Hindricks G. et al., 2020; Knuuti J. et al., 2020). Diagnosis CAD was confirmed by history of coronary arteries stenotic changes during invasive coronarography. AF paroxysm was checked by resting 12 leads electrocardiography. Criteria of exclusion were: valvular atrial fibrillation, heart failure (HF) from Class III to IV (by New York Heart Association), reported malignancies, chronic kidney disease (Glomerular Filtration Rate, GFR < 60 mL/min), thyroid pathlogy, inflammatory bowel disease, irritable bowel syndrome, pregnancy, taking probiotics and antibiotics for a month before the study. There were no vegetarians or vegans among the examined. All patient had HF stage B or C (McDonagh T. et al., 2023). The study was conducted at the base and was approved by the ethical commission of the Kiev City Clinical Hospital No. 12. Informed consent was obtained from all subjects in accordance with the Declaration of Helsinki and ethical commission submission. Baseline characteristics of investigated groups are performed in table 1.

Fecal SCFA was checked by gas chromatography with mass electron detection. We determined nine fatty acids in the collected samples – acetic acid (C2:0), propionic acid (C3:0), butyric acid (C4:0), isobutyric acid (C4:1), valeric acid (C5:0), isovaleric acid (C5:1), caproic acid (C6:0), isocaproic acid (C6:1) and caprylic acid (C8:0). These fatty acids include saturated (SFA) – acetic (C2:0), propionic (C3:0), butyric (C4:0), valeric (C5:0), caproic (C6:0), caprylic (C8:0) acids; and unsaturated (USFA) – isobutyric (C4:1), isovaleric (C5:1), isocaproic (C6:1) acids. Middle chain fatty acids (MCFA) include caproic acid (C6:0), isocaproic acid (C6:1) and caprylic acid (C8:0) (Michelle SW et al., 2019). Cardiometabolic risk factors which was explored are: total cholesterol (TC), tryglicerides (TG), low density lipoproteins (LDL), high density lipoproteins (HDL), lipoproteins α (Lpα), apolipoprotein A1 (ApoA1), apolipoprotein B (ApoB), C-reactive protein (CRP), interleukine – 6 (IL-6), TMA and TMAO (Lizogub V.G. et al., 2019, Li J.J. et al., 2022). Results were presented as mean ± standard error.
Table I. Baseline characteristics of investigated groups, mean ± standard error

<table>
<thead>
<tr>
<th>Characteristic / group</th>
<th>I group</th>
<th>II group</th>
<th>CG</th>
<th>P1-2</th>
<th>P2-3</th>
<th>P1-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>67.71±3.90</td>
<td>67.96±0.94</td>
<td>56.25±2.18</td>
<td>P&gt;0.05</td>
<td>P&gt;0.05</td>
<td>P&gt;0.05</td>
</tr>
<tr>
<td>Men (%)</td>
<td>48.99</td>
<td>47.97</td>
<td>48.15</td>
<td>P&gt;0.05</td>
<td>P&gt;0.05</td>
<td>P&gt;0.05</td>
</tr>
<tr>
<td>History of myocardial infarction (%)</td>
<td>30.87</td>
<td>26.02</td>
<td>0</td>
<td>P&gt;0.05</td>
<td>P&lt;0.05</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>History of stroke (%)</td>
<td>8.72</td>
<td>8.13</td>
<td>0</td>
<td>P&gt;0.05</td>
<td>P&lt;0.05</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>18.12</td>
<td>14.63</td>
<td>0</td>
<td>P&gt;0.05</td>
<td>P&lt;0.05</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>Obesity</td>
<td>8.84</td>
<td>12.0</td>
<td>0</td>
<td>P&gt;0.05</td>
<td>P&lt;0.05</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.02±0.33</td>
<td>26.93±0.43</td>
<td>28.12±2.10</td>
<td>P&gt;0.05</td>
<td>P&gt;0.05</td>
<td>P&gt;0.05</td>
</tr>
<tr>
<td>Smoking (%)</td>
<td>51.01</td>
<td>41.46</td>
<td>40.74</td>
<td>P&gt;0.05</td>
<td>P&gt;0.05</td>
<td>P&gt;0.05</td>
</tr>
<tr>
<td>Uric acid (mmol/l)</td>
<td>380.5±28.16</td>
<td>404.9±36.11</td>
<td>310.2±29.12</td>
<td>P&gt;0.05</td>
<td>P&lt;0.05</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>Total bilirubin (mmol/l)</td>
<td>11.3±0.09</td>
<td>12.4±0.08</td>
<td>11.7±0.11</td>
<td>P&gt;0.05</td>
<td>P&gt;0.05</td>
<td>P&gt;0.05</td>
</tr>
<tr>
<td>GFR (ml/min)</td>
<td>62.03±2.31</td>
<td>67.73±1.98</td>
<td>84.01±5.48</td>
<td>P&gt;0.05</td>
<td>P&lt;0.05</td>
<td>P&lt;0.05</td>
</tr>
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or [95% confidence interval (CI)] for continuous variables or as a number for categorical variables. Variables distribution for normality were checked by the Pearson criterion. Data were compared by Scheffe's or Dann multiple comparison methods depends with two critical regions for variables distribution respectively; Spearman's rank correlation coefficient was detected. ROC-curves with area under ROC-curve (AUC) calculation for gut microbiota component and their combinations were built for I and II groups (Faizi et all., 2023; Mandrekar JN, 2010). All calculations were done in MATLAB R2014a (License number 271828).

Results
Fecal SCFA composition was studied in all investigated groups. Total amount of fecal SCFA in I (71.13%) and II (41.89%) patients’ groups is significantly decreased in comparison with CG. Also, increasing of acetic (62.35%) and decreasing butyric (92.21%), valeric (72.36%), caprylic (99.84%) acids levels in 2 group in comparison with CG was found. In the I group was found significant arising of isovaleric (62.35%) and abundance of butyric (63.36%), valeric (97.75%), caproic (93.39%) acids in comparison with CG. In the II group significant increasing of valeric (1128.43%) and decreasing butyric (78.75%), isovaleric (56.29%), caprylic (99.21%) acids levels was revealed in comparison with I group. Isocaproic and isobutyric fecal acids were absent into the CG samples, but they appeared in I and II groups patients’ tests. Results are shown in figure 1.

Fig. 1. Fecal short chain fatty acids in investigated groups, mg/g
Moreover, in II group was found significant increase of USFA (485.44%) and decrease of MCFA (66.04%) levels in comparison with CG. In I group was found significant growth of USFA (258.54%) and decreasing of MCFA (98.49%) in comparison with CG. Also, in II group was significant decreasing MCFA (95.54%) and USFA (38.76%) levels in comparison with I group. Results are shown in figure 2.

Secondary, lipid profile, inflammatory markers and TMA, TMAO levels of investigated groups were evaluated. In the I and II group was a significant increasing of TC (32.64% and 43.06% respectively), TG (80.36% and 55.36% respectively), LDL (70.78% and 72.73% respectively), Lp(a) (41.17% and 54.95% respectively), ApoB (85.12% and 140.50% respectively), CRP (136.26% and 232.97% respectively), IL-6 (65.22% and 103.11% respectively), TMA (22.50% and 42.25% respectively), TMAO (50.00% and 136.31% respectively) and decreasing HDL (16.09% and 29.31% respectively) compared with CG.

In the II group significant increase of ApoB (29.91%), CRP (40.93%), IL-6 (22.93%), TMA (16.13%), TMAO (57.54%) levels were detected in comparison with a I group. Results are shown in table 2.
Further, heatmap correlation matrices were generated between lipid profile and fecal SCFA levels. The largest amount of correlations was checked between fecal SCFA composition and such clinical characteristics as TMAO (total number = 8), TMA (total number = 7) and CRP (total number = 6) levels. At the same time, the highest amount of correlations was between total amount of SCFA (total number = 9), MCFA (total amount = 7), butyric acid (total number = 6) and cardiometabolic risk factors. It is shown in figure 3.

ROC-analysis was done for each SCFA for better understanding their diagnostic value in pathogenesis of AF paroxysm in CAD patients. We calculated AUC for each sign. Results are shown in table 3.

AUC was more then 0.7 (acceptable) was found in three sings: total amount of SCFA (AUC = 0.7907) and butyric acid (AUC = 0.7727). They are shown in figure 4.

**Discussion**

SHFA are mainly produced by such probiotics as Roseburia, Eubacterium rectale, Blautia and Ruminococcus from dietary plant polysaccharides. Lack of this species leads to
impaired intestinal mucosal barrier function and increased bacterial endotoxin secretion, which were directly correlated with host metabolic and inflammatory disorders (Xiao S. et al., 2019; Patterson E. et al., 2016). SCFA cardioprotective effect is based on modulation T regulatory cell amount. Also, decreasing of total SCFA concentration is common foe diabetes, arterial hypertension, nonalcoholic steatohepatitis formation (Mandrekar JN, 2010). So, in general SCFA concentration is very important for gut microbiota stability (Lizogub V.G. et al., 2019).

Different SCFA are different in their role and tissue distribution. Butyrate is the main energy resource for colonocyte. Propionate activate liver gluconeogenesis. In the host organism they available to inhibit host histone deacetylases, which takes part in the protein’s synthesis (Coppola S. et al., 2021). Furthermore, different microbes produce different SCFA. For example, butyrate is mainly produced by Gramm positive microorganisms, as Firmicutes, acetate and propionate – by Gramm negative microorganisms, as Bacteroides. The type of SCFA production depends of different factors, including diet, gut microbiota composition, species evolution and colonic environment. After colonocytes absorb SCFA and they coming into blood flow. These SCFA can be used for carbohydrates and lipids synthesis of like cytokines for metabolism regulation. SCFA are able to activating brown adipose tissue, regulating liver mitochondrial function, maintaining body energy homeostasis, controlling appetite and sleep (He, J. et al., 2020).

SCFA plays essential role in lipids metabolism. By the latest data butyrate increase oxidation of fatty acids in brown adipose tissue, reduce the size of adipose cells, regulate activity of transcription factors, what leads for decreasing levels of triglycerides and fatty acids (He, J. et al., 2020; Schoeler M. et al., 2019). All of this confirmed the importance of SCFA and especially butyric acid in CAD pathogenesis. By literature data total amount SCFA decrease is associated with CAD presence (Lizogub V.G. et al., 2019).

Role of SCFA in AF pathogenesis is still undoubted, but there is a multiplicity of data about their role in AF risk factors pathogenesis (Hu, T. et al., 2022; Gawalko M. et al., 2022). So, SCFA further investigations are promising for new AF risk factors investigation and correction.

Conclusions
Fecal short chain fatty acids changes in patients with atrial fibrillation paroxysm and coronary artery disease were found in our investigation:
1. Isovalproic and isobutyric fecal acids appears in coronary artery disease and atrial fibrillation patients’ samples in comparison with control group.
2. In the patients with atrial fibrillation and coronary artery disease significant increasing of valeric (1128,43%) and decreasing butyric (78,75%), isovaleric (56,29%), caprylic (99,21%) acids, medium chain fatty acids (95,54%) and unsaturated fatty acids (38,76%) levels was revealed in comparison with coronary artery disease patients without arrhythmias (P<0,05).
3. The largest amount of correlations was between total amount of short chain fatty acids, medium chain fatty acids (total amount = 7), butyric acid (total number = 6) and cardiometabolic risk factors (P<0,05).
4. The acceptable role of total amount of short chain fatty acids (AUC = 0.7907) and butyric acid (AUC = 0.7727) in AF paroxysm occurrence in CAD patients was proven by ROC-analysis.

Perspectives of subsequent scientific research
To propose the new ways of gut microbiota and cardiometabolic risk factors correction will be interesting for future investigations.

Financing
This study did not receive external funding. The study was done according the department scientific research work "Changes in protein, carbohydrate and lipid metabolism in patients with coronary heart disease and arterial hypertension with heart rhythm disorders, possibilities of drug correction" 2021-2023 (state registration number 0121U108875)

Conflicts of Interest
it is no conflict of interest to declare.

Consent to publication
Informed consent was obtained from all subjects in accordance with the Declaration of Helsinki and ethical commission submission.
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Роль фекальних коротколанцюгових жирних кислот в патогенезі пароксизму фібриляції передсердь у хворих на ішемічну хворобу серця

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Анотація: склад кишкової мікробіоти та її метаболіти є важливою складовою здоров'я людини. Коротколанцюгові жирні кислоти (КЛЖК) є відомими метаболітами кишкової мікробіоти. Їх нестача характерна для дисліпідемії та запальніх змін. Але їх роль у патогенезі фібриляції передсердь (ФП) та ішемічної хвороби серця (ІХС) досі не вивчена. Мета: оцінити зміни коротколанцюгових жирних кислот у фекаліях у пацієнтів з пароксизмом ФП та ІХС та встановити їх зв'язок з відомими кардіометаболічними факторами ризику. Матеріали і методи: Обстежено 300 хворих. Ми розподілили їх на 3 групи: І група – 149 хворих на ІХС без порушень ритму, ІІ група – 124 пацієнти з ІХС та пароксизмом ФП та контрольна група (КГ) – 27 пацієнтів без ІХС та аритмій. Фекальні КЛЖК визначали за допомогою газової хроматографії з мас електронною детекцією. Результати: У нашому дослідженні виявлено зміни вмісту КЛЖК у калі у пацієнтів з пароксизмом ФП та ІХС. Ізокапронова та ізомасляна фекальні кислоти виявляються у зразках хворих на ІХС та ФП порівняно з КГ. У хворих на ФП та ІХС суттєво підвищувався вміст валеріанової (1128,43%) та знижувався масляної (78,75%), ізовалеріанової (56,29%), каприлової (99,21%) кислот, середньоланцюгових жирних кислот (95,54%) та ненасичених жирних кислот (38,76%) порівняно з хворими на ІХС без аритмій (Р<0,05). Найбільша кількість кореляцій була між загальною кількістю КЛЖК, середньоланцюгових жирних кислот (загальна кількість = 6) і кардіометаболічними факторами ризику (Р<0,05). ROC-аналізом доведено важливу роль загальної кількості КЛЖК (AUC = 0.7907) та масляної кислоти (AUC = 0.7127) у виникненні пароксизмі ФП у хворих на ІХС. Висновки: У хворих на пароксизмі фібриляції передсердь та ішемічну хворобу серця виявлено порушення синтезу коротколанцюгових жирних кислот. Запропонувати нові способи корекції кишкової мікробіоти та кардіометаболічних факторів ризику буде цікаво для майбутніх досліджень.

Ключові слова: ішемічна хвороба серця, фібриляція передсердь, жирні кислоти, кардіометаболічні фактори ризику.