Serological short-chain fatty acid and trimethylamine N-oxide microbial metabolite imbalances in young adults with acute myocardial infarction

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A B S T R A C T

Acute myocardial infarction (AMI) is associated with systemic inflammatory processes and metabolic alterations. Microbial-derived metabolites, such as short-chain fatty acids and trimethylamine N-oxide (TMAO), have emerged in recent years as key players in the modulation of inflammation, with potential implications for cardiovascular diseases. We performed a prospective observational study that monitored the serological concentration of bacterial metabolites in 45 young patients (<55 years) without cardiovascular risk factors but with AMI, at hospital admission and at 3 months of follow-up, and compared them with a control group. TMAO and acetate levels were significantly higher in AMI, whereas butyrate and propionate were significantly lower. The acetate/propionate ratio showed the most discrimination between AMI and controls by receiver operating characteristic analysis (area under the curve 0.769, $P < 0.0001$). A multivariate logistic regression model revealed that this ratio was independently associated with AMI. Short-chain fatty acid concentrations, but not TMAO, exhibited significant correlations with inflammatory and coagulation parameters. Three months after the acute AMI event, all metabolite levels returned to those observed in healthy controls except butyrate. In conclusion, our study reveals disturbances of the serological concentration of microbiota-derived metabolites in AMI that are also related to inflammatory and coagulation parameters. These findings highlight an interesting field of study in the potential role of microbial metabolites from gut in cardiovascular disease.

1. Introduction

Acute myocardial infarction (AMI) is a life-threatening manifestation of ischemic heart disease and is one of the main causes of

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Table 1
Demographic and baseline characteristics of AMI patients and healthy controls according to their hospital needs and disease severity.

	Controls (n = 36)	AMI patients (n = 45)	P-value
Age – years	48.17 ± 5.28	48.49 ± 5.5	0.790
Sex, male – n (%)	32 (88.9)	38 (84.4)	0.629
Weight – kg	78.24 ± 9.97	80.72 ± 13.19	0.367
BMI	26 ± 2.64	27.66 ± 4.07	0.207
Comorbidities			
Hypertension	6 (16.7)	9 (20)	0.701
Diabetes Mellitus	1 (2.78)	4 (8.89)	0.256
Dyslipidemia	13 (36.1)	26 (57.8)	0.052
Autoimmune disorder	4 (11.1)	4 (8.89)	0.739
Current smoker	13 (36.1)	27 (60)	0.028*
Heavy drinker	8 (22.2)	6 (13.3)	0.293
Cholesterol – mg/dL	210.13 ± 29.22	196.12 ± 49.77	0.133
HDL – mg/dL	50.13 ± 9.61	40.326 ± 23.81	0.034*
LDL – mg/dL	131.16 ± 30.24	132.67 ± 49.19	0.871
Triglycerides – mg/dL	149.61 ± 114.46	149.19 ± 74.05	0.985
Creatinine – mg/dL	0.893 ± 0.144	0.995 ± 0.322	0.063
Haemoglobin – g/dL	15.60 ± 1.13	14.75 ± 2.06	0.021*
Haematocrit – %	47.4 ± 3.16	44.73 ± 6.63	0.022*
MCV – fL	91.8 ± 5.76	90.27 ± 11.16	0.457
ESR – mm/h	5.714 ± 3.94	23.219 ± 26.14	0.004**
CRP – mg/L	1.69 ± 1.57	37.38 ± 55.63	<0.001***
C3 – mg/dL	112.63 ± 16.03	129.67 ± 29.11	0.002**
C4 – mg/dL	25.62 ± 6.94	30.3 ± 8.51	0.12*
Prothrombin G20210A (rs1799963) variant	1 (2.72)	1 (2.22)	0.999
Leukocytes – 10^3/μL	7.32 ± 1.6	10.28 ± 3.23	<0.001***
Neutrophils – 10^3/μL	3.82 ± 0.95	7.08 ± 2.1	<0.001***
Lymphocytes – 10^3/μL	2.69 ± 0.73	2.12 ± 1.01	0.006**
Monocytes – 10^3/μL	0.533 ± 0.16	0.878 ± 0.42	<0.001***
Eosinophils – 10^3/μL	0.219 ± 0.13	0.156 ± 0.118	0.023*
Basophils – 10^3/μL	0.048 ± 0.025	0.045 ± 0.029	0.697
Platelets – 10^3/μL	240.94 ± 69.6	220.96 ± 57.9	0.162

Data are expressed as mean ± SD or number (percentage). BMI, Body mass index; C3, complement component 3; C4, complement component 4; CRP, C-Reactive protein; ESR, Erythrocyte sedimentation rate; HDL, High-density lipoprotein; MCV, medium corpuscular volume; LDL, Low-density lipoprotein.

*, $P < 0.05$; **$P < 0.01$; ***$P < 0.001$ in Chi-square or unpaired t tests.

cardiovascular morbidity and mortality worldwide. The physiopathology of AMI is not the simple consequence of an atherosclerotic plaque rupture; the contribution of local and systemic cellular inflammation is now well accepted [1–3]. In the 1990s, experimental studies revealed that the ischemic area is significantly smaller when leukocyte-mediated inflammation is abrogated [4,5], and acute reactants such as C-reactive protein (CRP) are robust prognostic markers [6,7]. Anti-inflammatory treatments are currently being evaluated in AMI [8–10], although their efficacy is still being discussed [11,12].

Human-associated microbiota, particularly those located in the digestive tract, have emerged as a major regulator of systemic inflammatory and immune response. This ecosystem's composition has been redefined using culture-independent techniques [13], associating particular compositions with several inflammatory contexts [14–16], as well as in AMI compared with healthy controls [17,18]. Beyond the composition, the ecosystem's metabolic impact needs to be deciphered using metabolites of exclusive bacterial production, such as short-chain fatty acids (SCFAs) and trimethylamine N-oxide (TMAO) [13,19,20].

SCFAs can restore the imbalances in lipid and glucose metabolism and can thereby contribute to preventing and treating cardiovascular disease [21]. Although these compounds are enriched in the intestinal lumen, they reach the peripheral blood, triggering effects in the endothelium and distal organs [22]. Overall lower concentrations of SCFAs in plasma have been shown to increase cardiovascular risk in young adults [23,24], whereas butyrate and propionate have demonstrated anti-inflammatory properties by histone deacetylase inhibition [25–28].

TMAO is a microbiota-derived metabolite from dietary phosphatidylcholine which is particularly abundant in red meat and egg yolks. Evidences suggest TMAO plays an important role in AMI, in fact plasmatic TMAO has been identified as a predictor of and cardiovascular diseases (CVD) in different cohorts [29–31]. Moreover, this compound has also been associated with subclinical myocardial damage, being correlated with high-sensitivity cardiac troponin and severity in heart failure [32,33]. Experiments in germ-free mice confirmed a critical role for gut microbiota TMAO production in foam cell formation by controlling macrophage scavenger receptor expression [31]. Further evidences also pointed to the NLRP3 inflammasome activation leading to endothelial dysfunction [34]. Other diseases associated to TMAO levels includes neurological disorders, diabetes mellitus and chronic kidney disease [35,36].

The current study determined serological concentrations of TMAO and 3 main SCFAs (acetate, butyrate, and propionate) in a cohort of young patients with no cardiovascular risk factors during their hospital admission for AMI and 3 months after and compared with a control group. We also explored the possible correlation of these compounds with inflammation, thrombosis, and coagulation
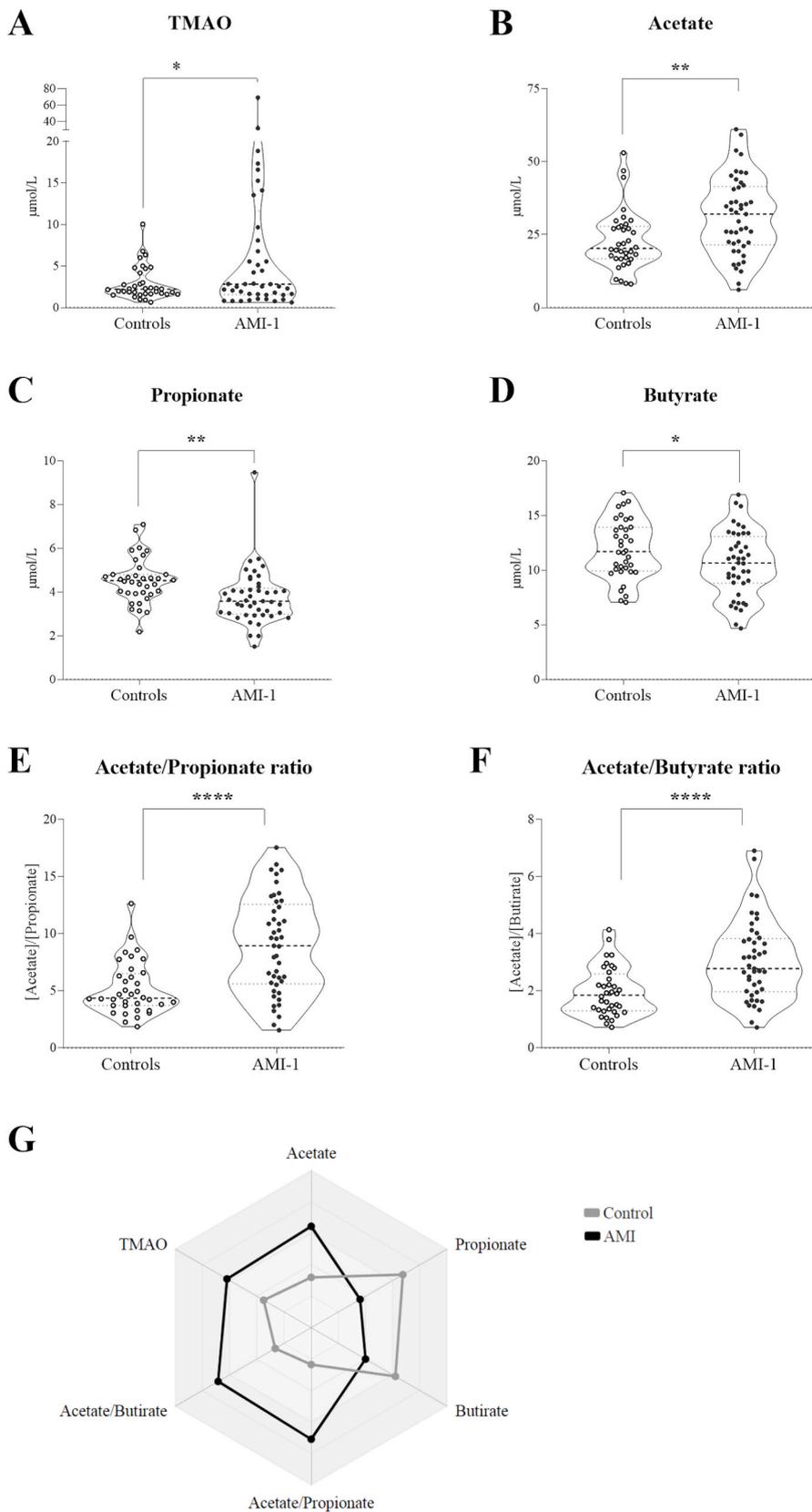
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Fig. 1. Microbial metabolite levels exhibit alterations in AMI patients' serum compared with healthy controls. Serum levels of TMAO (**A**), acetate (**B**), propionate (**C**), butyrate (**D**) and acetate/propionate (**E**) and acetate/butyrate ratio (**F**). **G**: Radar plot with the mean z-score values for each analyzed variable in control (grey line) and AMI patients (black line). *, $P < 0.05$; **, $P < 0.01$; ****, $P < 0.0001$ in unpaired t-test.

parameters.

2. Materials and methods

Study design and patient recruitment. This prospective study recruited participants aged 18–55 years (n = 45) admitted to our center's Coronary Unit with AMI, with or without ST segment elevation, between October 2018 and March 2021. The exclusion criteria were a previous diagnosis of antiphospholipid syndrome; acquired or hereditary thrombophilia; chronic myeloproliferative syndromes; anatomical vascular obstructions; thrombotic thrombocytopenic purpura; active sepsis; recent prolonged immobilization (>6 months); or death within the first 3 months after the AMI episode. The recruited patients' baseline clinical characteristics are shown in Table 1. Each patient contributed a baseline blood sample at admission, and 20 patients provided an additional blood sample after 12–15 weeks. Relevant data related to cardiovascular risk factors were recovered from the clinical chart, as well as other variables related to coagulation disorders. A control group of healthy volunteers (n = 36) undergoing a routine laboratory analysis with the same demographic characteristics was recruited from primary care. The Strengthening the Reporting of Observational Studies in Epidemiology checklist was followed for writing the manuscript.

Ethics statement. The study was conducted in accordance with the Declaration of Helsinki and was approved by the institutional review board from Ramón y Cajal Hospital under the code 184/18. Signed informed consent was obtained from all participants.

Sample processing. Pre-analytical processing of the samples from the patients included in this study was performed by Biobank Hospital Ramón y Cajal-IRYCIS (National Registry of Biobanks B.0000678), integrated within the Biobanks and Biomodels Platform of the ISCIII (PT20/00045). Samples were processed following standard operating procedures, with appropriate approval from the relevant ethics and scientific committees. The patients' serum samples were immediately aliquoted and stored at $-80\ °C$, then defrosted slowly for 24 h at $-20\ °C$ and a further 24 h at $4\ °C$.

Microbial-derived metabolite determination. Serum levels of TMAO and SCFAs were determined following a previously described protocol at the Center of Metabolomics and Bioanalysis (CEMBIO) Laboratory (https://cembio.uspceu.es/) [37]. Briefly, for TMAO determination, D9 deuterated TMAO (Cambridge Isotope Laboratories, Andover, MA, USA) was used as internal standard. Before analysis, 20 µL of each sample were mixed with 20 µL of D9 TMAO (10 µM in MeOH) and 60 µL of MeOH, vortexed and subsequently centrifuged (10 min, $4\ °C$, 13,000 g) and the supernatants were transferred to analytical vials. The analysis was carried out using a liquid chromatography system (1290 Infinity, Agilent Technologies, Santa Clara, CA, USA) with XBridge® BEH Amide column (100 × 2.1 mm, 2.5 µm, Waters, Milford, MA, USA) coupled to a triple quadruple mass spectrometer (6460, Agilent Technologies) in MRM positive mode. Whereas SCFAs (acetate, butyrate, and propionate) were determined by gas chromatography (Agilent 7890 A Series) using hydrogen as carrier gas, a capillary BP-21 column (SGE, Cromlab SL, Barcelona, Spain) coupled to a flame ionization detector (FID) and 4-methylvaleric acid as an internal standard (Sigma-Aldrich). SCFAs peaks were identified according to the retention time of standard compounds (Sigma-Aldrich, ST. Louis, MO, USA).

Statistical Analysis. The data are presented as means and standard deviations, and the differences between groups were evaluated with the t-test for continuous variables, a chi-squared test for dichotomous variables, or an analysis of variance model followed by the Tukey–Kramer test for those variables with more than 2 categories. Associations among SCFA levels and parameters of inflammation, thrombosis, and coagulation were evaluated by Spearman's correlation tests. A receiver operating characteristic (ROC) curve analysis was used to determine the capacity of SCFA levels to discriminate between the groups. Optimal cutoff values were estimated by the Youden index.

Univariate regression models were performed (Supplementary Table 1) previously to a Wald forward stepwise multivariate regression model for test independent association with AMI of several parameters including blood counts (leukocytes, neutrophils, lymphocytes, monocytes, eosinophils, basophils, and platelets), inflammation related markers including CRP, erythrocyte sedimentation rate (ESR), and complement components C3 and C4, and bacterial metabolites: TMAO, acetate, propionate, butyrate, acetate/propionate ratio and acetate/butyrate ratio. Summary of steps in logistic regression model (Supplementary Table 2). P-values of less than 0.05 were considered significant, all P-values were 2-sided, and 95 % confidence intervals (95 % CI) are presented. Statistical analyses were conducted using Prism 8.0 (GraphPad) and SPSS version 23 (IBM) software.

3. Results

Clinical characteristics and microbial metabolite levels in baseline AMI blood samples. Other than a slightly lower high-density lipoprotein level and a higher smoking frequency in the patients, there were no significant differences for age, sex, weight, body mass index, comorbidities, cholesterol, or triglycerides between the groups (Table 1). In contrast, deep leukocyte abnormalities accompanied by high levels of acute-phase CRP, the complement components C3 and C4, and the ESR revealed an inflammatory state in the patients with AMI (Table 1). Regarding microbial metabolites, the AMI group had significantly higher serum TMAO ($P = 0.014$) (Fig. 1A) and acetate levels ($P = 0.003$) (Fig. 1B); however, propionate and butyrate were significantly lower in the patients with AMI ($P = 0.004$ and $P = 0.035$) (Fig. 1C and D). SCFA ratios including acetate/butyrate and acetate/propionate were also higher in those with AMI ($P < 0.0001$) compared with the healthy controls (Fig. 1E–G).
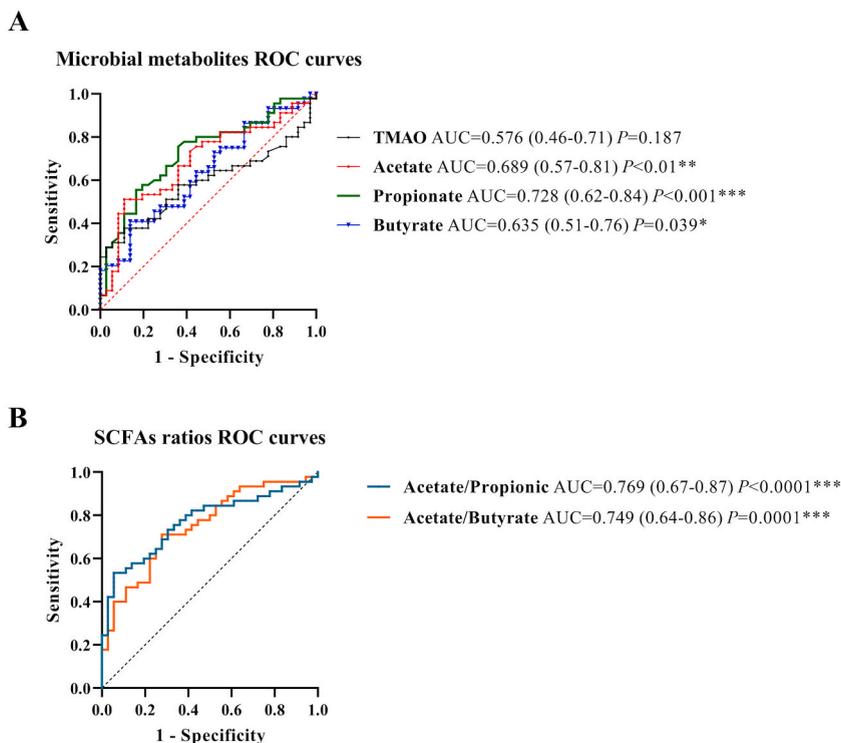
A

Microbial metabolites ROC curves

- **TMAO** AUC=0.576 (0.46-0.71) *P*=0.187
- **Acetate** AUC=0.689 (0.57-0.81) *P*<0.01**
- **Propionate** AUC=0.728 (0.62-0.84) *P*<0.001***
- **Butyrate** AUC=0.635 (0.51-0.76) *P*=0.039*

B

SCFAs ratios ROC curves

- **Acetate/Propionic** AUC=0.769 (0.67-0.87) *P*<0.0001***
- **Acetate/Butyrate** AUC=0.749 (0.64-0.86) *P*=0.0001***

Fig. 2. ROC curves for AMI and control discrimination. ROC curves for AMI patient identification of microbial metabolites (**A**) and SCFAs ratios (**B**) are shown. Area under the curve (AUC) with 95 % confidence interval are shown. *, $P < 0.05$; **, $P < 0.01$; ****, $P < 0.0001$ in AUC/ROC analysis.

Table 2
Cut-off values of microbial metabolites and ratios for AMI patient's identification.

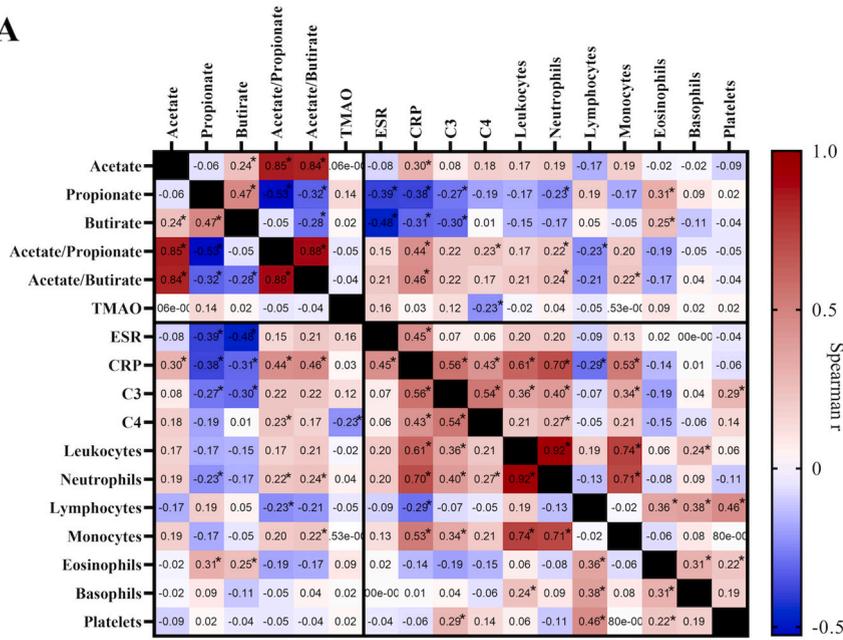| Molecule	Cut-off value in μmol/L	Youden index	Sensitivity	Specificity
TMAO	>7.440	0.262	0.289 (0.177–0.434)	0.973 (0.862–0.999)
Acetate	>31.55	0.400	0.511 (0.370–0.650)	0.889 (0.747–0.956)
Propionate	<4.175	0.395	0.756 (0.613–0.858)	0.639 (0.476–0.775)
Butyrate	<9.680	0.270	0.409 (0.277–0.556)	0.861 (0.713–0.939)
Acetate/Propionate ratio	>8.732	0.478	0.533 (0.391–0.671)	0.944 (0.818–0.990)
Acetate/Butyrate ratio	>2.213	0.433	0.7111 (0.566–0.823)	0.722 (0.560–0.842)

Cut-off values were estimated from ROC curves by Youden index. Values of sensitivity and specificity (95% confidence interval) for each cut-off value are shown.

In the area under the curve (AUC)/ROC curve analysis, propionate was the main metabolite discriminating patients with AMI from controls (AUC 0.728, $P < 0.001$), and all the SCFA ratios improved the AUC compared with single metabolites (AUC 0.769 and AUC 0.749, respectively, $P < 0.0001$) (Fig. 2A and B). Given that one of the major problems for the clinical translation of SCFA determinations is the lack of cutoff values for normality, especially in serum/plasma samples. By using the Youden index, we estimated cutoffs for discerning control and AMI values with various grades of sensitivity and specificity (Table 2). There were no correlations between the bacterial metabolites and the clinical characteristics of the patients and controls, such as hypertension, dyslipidemia, and diabetes (Supplementary Fig. 1).
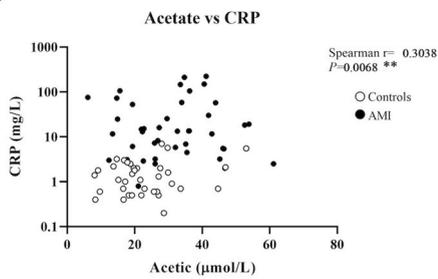
SCFA levels correlate with acute inflammation and immune parameters. As mentioned above, the patients with AMI exhibited certain abnormalities, such as neutrophilia, lymphopenia, high CRP, and a high ESR, which indicates their inflammatory status. Given that SCFAs are involved in the modulation of inflammation, we aimed to study the possible correlation of SCFA levels and inflammatory and immune parameters. There was an overall positive correlation with acetate but a negative association with butyrate and propionate (Fig. 3A). The most significant positive correlations were acetate with CRP, the acetate/propionate ratio with CRP, and the absolute neutrophil number. Negative values were obtained for propionate with both CRP and ESR, and butyrate with ESR (Fig. 3B–G). In contrast, TMAO exhibited no relevant correlation with inflammatory parameters (Fig. 3A).

Due to the large number of correlations between the parameters, we decided to test which variables presented an independent association with AMI. We therefore included 17 parameters with routine inflammatory markers and microbial metabolites in a multivariate logistic regression model. We found that the acetate/propionate ratio and the neutrophil and lymphocyte count were
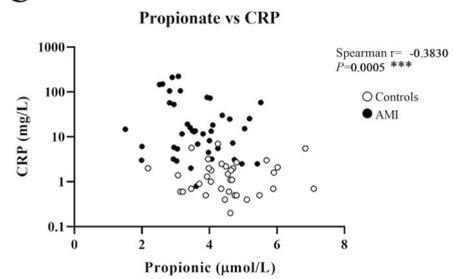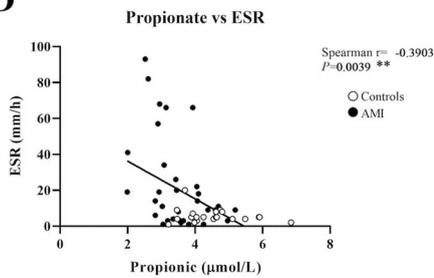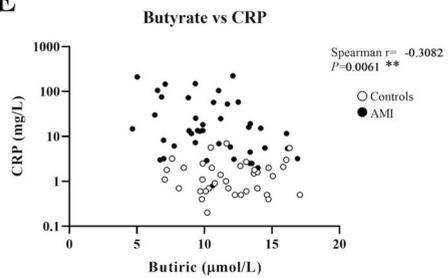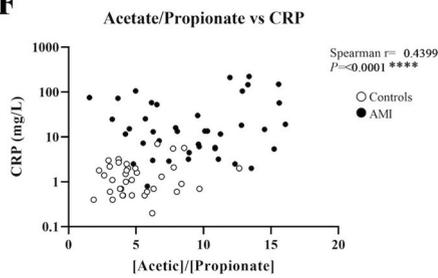
A

B Acetate vs CRP

C Propionate vs CRP

D Propionate vs ESR

E Butyrate vs CRP

F Acetate/Propionate vs CRP
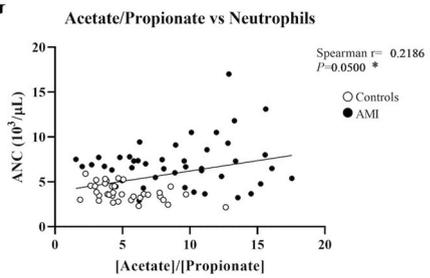

G Acetate/Propionate vs Neutrophils

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Fig. 3. SCFAs correlates with several inflammatory parameters. A. Correlation matrix heatmap including microbial metabolites, SCFA ratios and routine inflammatory-related parameters. Those with statistical significance defined as *P*-value<0.05 are labeled with * symbol. **B-G.** Some relevant correlation graphs including SCFAs and its ratios versus routine laboratory inflammation-related markers are shown. CRP, C reactive protein; ESR, erythrocyte sedimentation rate; ANC, absolute neutrophil count.

independently associated with AMI (Supplementary Table 2).

Microbial metabolite associations with coagulation parameters, cardiologic alterations, and other relevant parameters. Coagulation and thrombotic disorders are linked to inflammation and have an important role in AMI pathophysiology (Fig. 4A). It is important to highlight that propionate positively correlated with antithrombotic proteins such as antithrombin III and protein S, whereas there was a negative correlation for the international normalized ratio (INR) (Fig. 4B–D). Butyrate negatively correlated with Russell's Viper Venom assay (Fig. 4E). In contrast, the acetic/propionic ratio positively correlated with the assay and INR and negatively correlated with the antithrombotic protein S (Fig. 4F–G). Regarding SCFA levels and cardiac abnormalities in AMI, there were no clear differences in SCFA levels when classifying the patients according to AMI type, left-ventricular ejection fraction, or multi-vessel disease (Supplementary Fig. 2).

TMAO and SCFAs after AMI resolution. After demonstrating AMI, patients had abnormal levels of the microbial metabolites and SCFAs correlated with inflammatory markers; the remaining question is their behavior after the acute phase of myocardial infarction. Twenty of the patients provided a second blood sample at the 12-week follow-up. At 12 weeks after the AMI, the metabolite levels of the AMI group tended to reach values similar to those of the control group: decrease in TMAO (Fig. 5A), slight decrease in acetate (Fig. 5B) and increase in propionate (Fig. 5C); however, only butyrate and subsequently the acetate/butyrate ratio demonstrated a significant increase after the infarction resolved (Fig. 5D–F).

4. Discussion

Therapeutic strategies against the inflammatory response before and after AMI continue to be investigated, and the involvement of gut microbiota in human global homeostasis is evident. Studies investigating the gut microbiome in coronary artery disease and AMI have demonstrated evidence of dysbiosis in these patients [17,18,38,39]. However, results are hardly standardizable between studies, or lack parallel serum samples limiting their translation to the functionality, which is better reflected by circulating microbiota-derived metabolites [38]. In addition, microbiota composition analysis normally requires next-generation sequencing with subsequent complex and time-consuming bioinformatical analysis hampering its applicability in acute diseases. Beyond the taxonomic composition, the focus should include final metabolites, given that they are able to exert remote functions. The differences between patients and controls need to be validated in other cohorts, seeking second-stage strategies that modulate or block the production of these bacterial metabolites to control the global inflammation associated with AMI.

SCFAs are an exclusive bacterial metabolite normally derived from complex carbohydrate fermentation that has classically been measured in fecal samples [40]. Given that our interest was in their systemic effect, we decided to monitor their concentration in serum. The lack of knowledge of SCFA dynamics in serum, which probably presents a Gaussian-like curve like that of glucose and other dietary compounds, limits its evaluation. There is therefore an urgent need to establish normality criteria for SCFA determinations. In our study, we estimated the best cutoff values for AMI and control discrimination by the Youden index to suggest "normality ranges." Our most relevant result was the significant decrease in propionate and butyrate concentrations along with higher acetate levels in the patients with AMI. Propionate supplementation has been related to improved myocardial damage recovery in mouse animal models [41,42]; however, there is a lack of information regarding the overall effects of SCFAs [38,43].

SCFAs are supposed to have an overall anti-inflammatory effect [25,27]. Propionate and butyrate have histone deacetylase inhibitory activity [26,27] and are likely to also play a role in blood pressure control via host G-protein-coupled receptors [44]. In this study, we found correlations between SCFA levels and common inflammation markers such as CRP and ESR. Acetate correlated positively with these inflammatory markers, whereas propionate and butyrate exhibited negative correlations, revealing inflammatory association and suggesting compensatory actions among SCFAs. Microbial metabolites have been proposed as possible modulators of thrombotic events [45]. Our data indicated that propionate had a negative correlation with INR and a positive correlation with antithrombotic proteins such as antithrombin III and S protein.

The expected distribution of the main SCFAs, such as acetate (C2)/propionate (C3)/butyrate (C4), is 60 %/20 %/20 %, whereas other SCFAs represent less than 1 % of total abundance [46,47]. In the controls, we observed a distribution of 55 %/12 %/33 %, whereas during the AMI episode it was 70 %/9 %/21 % and, after recovery, 61 %/9 %/30 %, indicating that after resolution butyrate appears to approximate some proportion of "normality." To minimize the influence of sample processing and facilitate inter-laboratory standardization, we estimated different SCFA ratios, including acetate/butyrate and acetate/propionate [48]. The acetate/propionate ratio was significant for having the best ROC performance for AMI and control discrimination (AUC 0.769), significantly increasing the levels observed in acetate, propionate, and butyrate individually. Moreover, the logistic regression model (including 17 clinical and metabolic parameters) revealed an independent association between the acetate/propionate ratio and AMI, reinforcing the relevance of this ratio as a biomarker.

The possible contribution of TMAO to cardiovascular disease has recently been explored in a systematic review and meta-analysis [30]. The authors showed that high TMAO levels are associated with major adverse cardiovascular events and all-cause mortality [30]. In humans, patients with type 2 diabetes and chronic kidney disease have a higher proportion of TMA-producing microbiota and a positive correlation between TMAO and some biomarkers of inflammation and endothelial dysfunction [35]. Other reports showed a
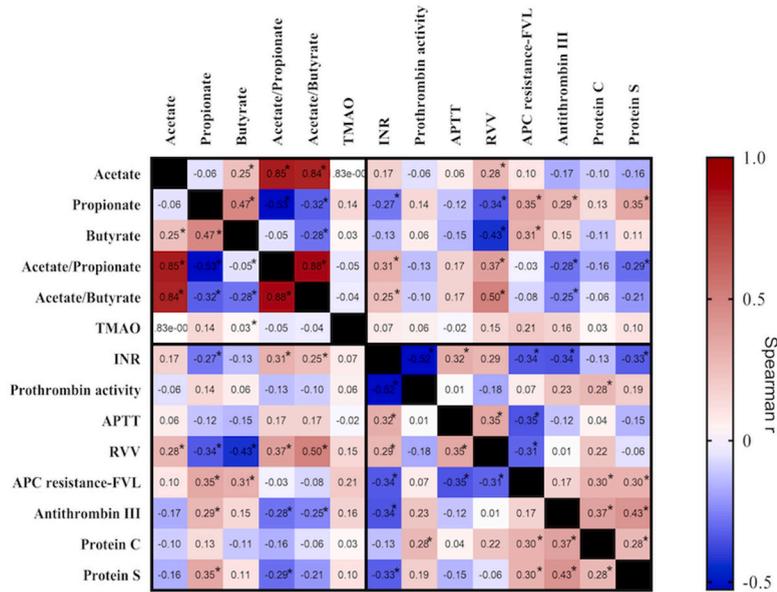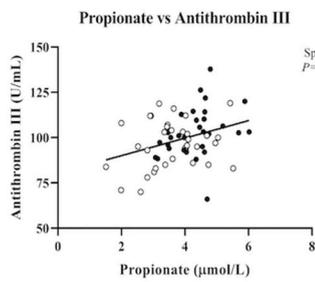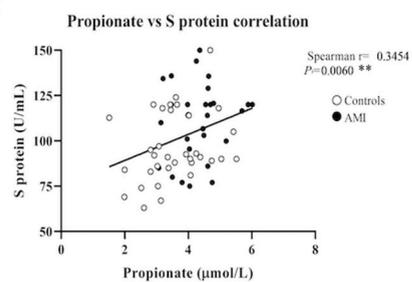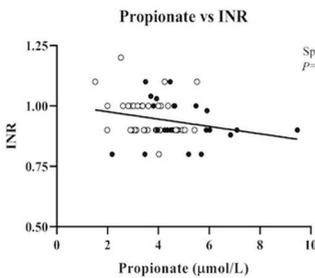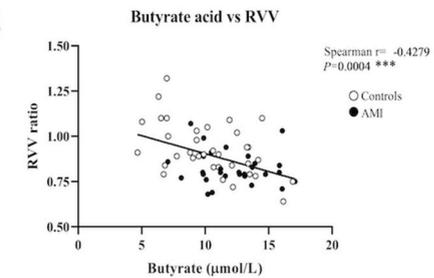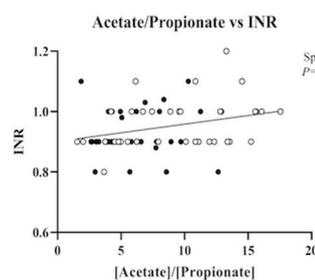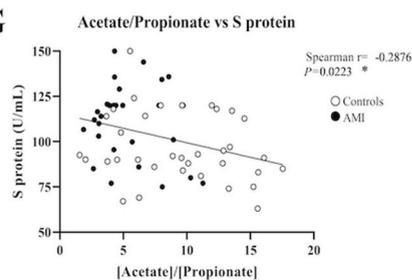
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Fig. 4. SCFAs correlates with several coagulation parameters. Correlation matrix heatmap including microbial metabolites, SCFA ratios and routine coagulation-related parameters. Those with statistical significance defined as *P*-value<0.05 are labeled with * symbol. **B-G.** Some relevant correlation graphs including SCFAs and its ratios versus routine laboratory coagulation-related markers are shown. APTT, activated partial thromboplastin time in seconds; APC resistance, Activated protein C resistance; RVV, Russell's Viper Venom ratio.
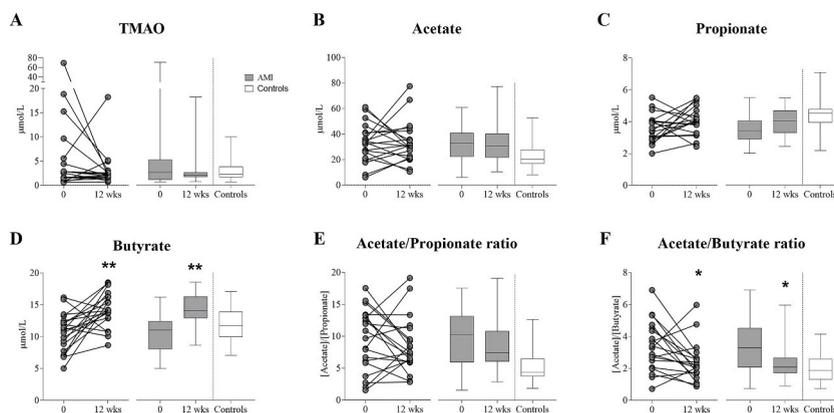

Fig. 5. Microbial metabolites levels in plasma after AMI resolution. Longitudinal samples after 12 weeks from AMI resolution were taken (n = 20). Plasma levels of TMAO (**A**), Acetate (**B**), Propionate (**C**), Butyrate (**D**), Acetate/Propionate (**E**) and Acetate/Butyrate (**F**) ratios are shown. *, $P <$ 0.05; **, $P <$ 0.01 in Wilcoxon paired *t*-test (left panel) and unpaired *t*-test (right panels) in 0 *versus* 12 weeks samples from AMI patients.

positive correlation between TMAO and ADMA (endothelial dysfunction biomarker) only in patients with HIV and type 2 diabetes, but found no correlation with high sensitivity C-reactive protein (hsCRP), which is an inflammatory biomarker [49]. In our cohort, TMAO was significantly increased in the patients with AMI at hospital admission; however, TMAO showed no correlation with inflammation or coagulation parameters. Although TMAO has been linked to CVD by several studies, our work indicates other microbial-derived metabolites such as acetate and propionate seem more interesting for AMI than TMAO.

A striking fact of this molecule is the wide dispersion of data in the patient group, suggesting the possibility of low and high subgroups of patients, a possibility that should be explored in future studies with larger cohorts, as well as determining its systemic role and involvement in cardiovascular disease.

An interesting open question is whether microbial alterations in plasma have a causal role or are just a consequence of AMI. Our longitudinal analysis revealed that SCFA and TMAO levels tended to reach normality after AMI resolution. We also found no differences in the levels of metabolites based on comorbidities, either in the patients with AMI or in the controls, which suggests that the imbalances observed at hospital admission in the patients with AMI could be due to the acute physiopathology of AMI and are not just markers of risk or derived from other chronic underlying diseases. Our chosen cohort are young adults (<55 years) with no patent cardiovascular risks or family background. Regarding genetics, one of the most frequent genetic risk factors for venous thrombo-embolism is the G20210A (rs1799963) polymorphism, which increased myocardial infarction risk in <55 years individuals [50,51]. In our cohort, only one of the included patients exhibited this alteration. Longer prospective longitudinal studies with larger cohorts and more detailed genetic background are needed to verify if SCFA disorders predispose to AMI or are caused by the infarction itself and its related inflammation.

Another important fact is that microbial metabolite levels in serum might be influenced by dietary intake, the potential bias of which we could not exclude. Fiber and omega-3-rich diets change the abundance of some gut microbial genera and decrease total and LDL cholesterol [52–55], which could lead to increased SCFAs production [56]. In our study, except for a slight decrease in HDL levels, we have not found differences in cholesterol and triglyceride levels between patients and healthy controls, suggesting no evident dietary discrepancies between them. Nevertheless, additional studies analyzing dietary changes would be necessary to deeply understand the interplay between diet, serum SCFAs levels and microbiota composition.

In this study, we described the microbial metabolite disorders in the serum of patients with AMI, which were mainly characterized by increased TMAO and acetate and decreased propionate and butyrate levels. We also estimated different SCFA ratios, showing greater differences between the patients with AMI and the controls than in the individual SCFAs. At hospital admission, SCFA levels correlated with inflammatory and thrombotic markers, an observation that also agrees with data after AMI resolution, in which SCFAs, especially butyrate, tended to reach control values. In conclusion, our study provides useful information on microbial metabolites in AMI and encourages the further study of the role of this type of compound in acute inflammatory-related diseases.

Data availability statement

Data will be made available on request to corresponding author.

Funding

CRediT authorship contribution statement

José Avendaño-Ortiz: Data curation, Formal analysis, Funding acquisition, Methodology, Writing – review & editing. **Álvaro Lorente-Ros:** Data curation, Investigation, Resources. **Andrea Briones-Figueroa:** Data curation. **Patricia Morán-Alvarez:** Resources. **Antia García-Fernández:** Resources. **Sandra Garrote-Corral:** Resources. **Irene Amil-Casas:** Resources. **Ángela Carrasco-Sayalero:** Resources. **Amalia Tejada-Velarde:** Resources. **Asunción Camino-López:** Resources. **Manuel Jiménez-Mena:** Resources. **Rosa del Campo:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Supervision, Writing – original draft, Writing – review & editing. **Lourdes Villalobos-Sánchez:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Writing – original draft, Writing – review & editing. **María Jesús García-Villanueva:** Formal analysis, Investigation, Writing – review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Rosa del Campo reports financial support was provided by Spanish Ministry of Health-Carlos III Health Institute (ISCIII).

Acknowledgements

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e20854.

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