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Role of major cardiovascular surgery-induced metabolic reprogramming in acute kidney injury in critical care

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Abstract

Purpose: Major cardiovascular surgery imposes high physiologic stress, often causing severe organ dysfunction and poor outcomes. The underlying mechanisms remain unclear. This study investigated metabolic changes induced by major cardiovascular surgery and the potential role of identified metabolic signatures in postoperative acute kidney injury (AKI).

Methods: A prospective observational study included 53 patients undergoing major cardiovascular surgery in 3 groups: cardiac surgery with cardiopulmonary bypass (CPB $n = 33$), without CPB ($n = 10$), and major vascular surgery ($n = 10$). For each patient, peripheral blood samples were collected pre-surgery, and at 6 h and 24 h post-surgery. Untargeted metabolomics using mass spectrometry quantified 8668 metabolic features in serum samples. Linear mixed-effect models (adjusted for age, sex, and body mass index) and pathway analyses were performed.

Results: In the cardiac surgery with CPB group, 772 features were significantly altered ($P < 2.8E-05$) across the 3 time points. These features were enriched in five classes, all related to protein metabolism, with glycine and serine metabolism being the most represented. Cardiac surgery with CPB showed a distinct metabolic signature compared to other groups. Patients who developed postoperative AKI exhibited increased protein catabolism (including valine, leucine, and isoleucine degradation), disruptions in the citric acid cycle, and plasmatic accumulation of acylcarnitines.

Conclusion: Major cardiovascular surgery, particularly with CPB, induces significant changes in protein metabolism. Patients developing postoperative AKI exhibited specific metabolic signatures. These findings may be critical for designing interventions to minimize organ dysfunction, including AKI, and improve outcomes in major cardiovascular surgery.

Keywords: Major cardiovascular surgery, Metabolism, Metabolomics, Amino acid, TCA cycle, Citric acid, Acylcarnitines, Acute kidney injury

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Background

Cardiovascular diseases are the leading cause of death worldwide, accounting for over 17.9 million deaths annually, i.e., 32% of all deaths [1, 2]. The burden is increasing, with up to one-third of patients potentially requiring surgical intervention during their lifetime [1]. Currently, over 2 million cardiac surgeries are performed globally each year.

Despite its crucial role in cardiovascular health, treating approximately 11% of global disability-adjusted life years [1], cardiac surgery carries substantial risks of morbidity and mortality. Postoperative recovery often involves an inflammatory host response, with myocardial ischemia, endothelial dysfunction, and ischemia–reperfusion injury contributing to varying degrees of organ dysfunction [3, 4]. However, we do not understand the molecular bases of postoperative stress responses, without which evidence-based improvement in outcomes of these patients may be out of reach.

Although cardiovascular energy metabolism has been studied since the 1930s, significant clinical correlations between metabolic changes and the onset or progression of various cardiovascular diseases have been found only recently. Current evidence suggests that disrupted metabolic pathways are part of a complex immunometabolic regulatory network affecting tissue hemostasis, reprogramming of cellular metabolism, and immune cell function [5].

State-of-the-art metabolomics methods [6–8] have the potential to provide a comprehensive characterization of metabolomic postoperative patterns, offering insights into metabolic signatures associated with surgical stress and postoperative organ dysfunction. Our long-term goal is to find novel interventions that improve the outcome of major cardiovascular surgery in critical care. To this end, in this study, we aimed to characterize the metabolic programs induced by surgical stress during major cardiovascular procedures and to find links between metabolic signatures and postoperative organ dysfunction, such as acute kidney injury (AKI), a frequent event in major cardiovascular surgery that leads to poor outcomes.

Take-home messages

Major cardiac surgery using CPB induces a distinct, strong, and specific metabolic signature characterized by an overrepresentation of protein metabolism. Some features of the identified pattern (including *O*-3-methylglutarylcarnitine) strongly predict acute kidney injury.

Methods

Study population and definition of outcomes

This study complied with the Declaration of Helsinki. It was approved by the review board of Hospital de Santa Maria, Unidade Local de Saúde Santa Maria (Ref. N.º 23/18), following the "Strengthening the Reporting of Observational Studies in Epidemiology" (STROBE) guidelines. All patients signed informed consent. We included consecutive patients older than 18 who underwent major cardiovascular surgery between August and November 2023. Patients were divided into three groups: (1) cardiac surgery with cardiopulmonary bypass ("CPB" group), (2) cardiac surgery without CPB ("No CPB" group), and (3) major vascular surgery ("VascSurg" group). Exclusion criteria were previous cardiac surgery, concomitant procedures, history of neoplasia, and ongoing immunomodulators medication. Based on our previous studies and independent literature reports on untargeted metabolomics in other conditions, we set a minimum analysis of 30 patients with surgery-induced organ dysfunction with CPB. In the same period, we also collected samples from patients who underwent cardiac surgery without CPB, fulfilling the inclusion criteria and an equal number of patients submitted to major vascular surgery. Full details regarding our study design and patients' inclusion are described in Supplementary Material 1. Peripheral blood samples were collected before the procedure, and then at 6 h and 24 h after surgery. The serum was collected in dry tubes, centrifuged, and stored at −80 ºC.

Postoperative acute kidney injury (AKI) was defined according to the KDIGO criteria [9]. Stage 1 was defined as an increase in the serum creatinine level of ≥ 0.3 mg/dl (26.5 µmol/L) within 48 h, at least 1.5 times baseline, or a urine output (UO) < 0.5 ml/kg/h for 6–12 h; stage 2 as an increase in serum creatinine of 2–2.9 times baseline or UO < 0.5 ml/kg/h for ≥ 12 h; stage 3 as serum creatinine increase 3 times baseline, increase in serum creatinine ≥ 4 mg/dl (≥ 353.6 µmol/L), initiation of renal replacement therapy, UO < 0.3 ml/kg/h for ≥ 24 h or anuria ≥ 12 h.

Untargeted metabolomics

After isolation of blood serum, proteins were removed by adding 400 µL of a methanol/ ethanol mixture (4:1, v/v) to 100 µL of serum in an Eppendorf tube, followed by vigorous vortex shaking for 5 min at room temperature and centrifugation at 4000×*g* for 10 min at 4 °C. The supernatant was carefully collected avoiding contamination with the precipitated proteins, transferred to another Eppendorf tube, shock frozen with liquid nitrogen, and stored at −80 °C until analysis. Extracted samples were thawed on ice, centrifuged for 2 min at 15,000×*g*, and diluted

according to the different sample weight with 0.1% formic acid (RP, reversed phase) or 50% acetonitrile (ACN) (HILIC, hydrophilic interaction chromatography). 2.5 µL of each diluted sample were pooled and used as a quality control (QC) sample. Samples were randomly assigned into the autosampler and metabolites were separated on a SeQuant ZIC-pHILIC HPLC column (Merck, 100 3 2.1 mm; 5 mm) or a RP-column (Waters, ACQUITY UPLC HSS T3 150 3 2.1; 1.8 mm) with a flow rate of 100 mL/min delivered through an Ultimate 3000 HPLC system (Thermo Fisher Scientific). The gradient ramp-up time is 25 min from 10 to 80% B in HILIC (A: ACN; B: 25 mM ammonium bicarbonate (ABC) in water) and from 1 to 90% B in RP (A:0.1% FA in water B:0.1% FA in ACN). Metabolites were ionized via electrospray ionization in polarity switching mode after HILIC separation and in positive polarity mode after RP separation. Sample spectra were acquired by data-dependent high-resolution tandem mass spectrometry on a Q-Exactive Focus (Thermo Fisher Scientific). The ionization potential was set to 3.5/−3.0 kV, the sheet gas flow was set to 20, and an auxiliary gas flow of 5 was used. Samples were analyzed in a randomized fashion and QC samples were additionally measured in confirmation mode to obtain additional MS/MS spectra for identification. Obtained datasets were processed by Compound Discoverer 3.0 (Thermo Fisher Scientific). Compound annotation was conducted by searching the mzCloud database with a mass accuracy of 3 ppm for precursor masses and 10 ppm for fragment ion masses as well as ChemSpider with a mass accuracy of 3 ppm using BioCyc, Human Metabolome Database, KEGG, MassBank and MetaboLights as databases.

Statistical analysis

Given the prospective nature of this study, no data was missing for the statistical analysis, and all the variables for each patient were collected. The statistical analysis was performed with R [10]. To investigate time-dependent metabolite changes in each group, we employed linear mixed-effect models with random participant-specific intercepts (Supplementary Material 2). Features (ion counts) were natural log-transformed and scaled within each time point and study group. Models were adjusted for age, sex, and body mass index, which are known to influence metabolite levels [11–14]. For multiple testing correction, we applied both Bonferroni (significant threshold P-value $< 2.77 \times 10^{-5}$ for 1803 features) and the less conservative Benjamini–Hochberg false discovery rate (FDR) correction (P value < 0.05). Nominal P value < 0.05 were also reported.

For the PCA (principal component analysis), the log fold change of the intensity between two time points was used. For the comparison of metabolites between groups, a generalized linear model was used, considering the metabolites with significant changes (FDR (false discovery rate) < 0.05 and a peak intensity variation superior to 50%). To build the metabolic networks, significantly changed metabolites (FDR < 0.05 and a peak intensity variation superior to 50%) were used, using the absolute value of log fold changes as input for the metabolic network. The propagation score for various metabolic pathways was analyzed to assess if it was significantly higher than expected by chance (test based on 5000 permutations, in which the log fold changes of the input are randomly assigned to various metabolites in the network and the propagation in the network is recalculated). High-logp (-log10 of the permutation test P value) values indicate that the pathway propagation score was significantly higher than would be expected by chance.

To assess the association between metabolites and the occurrence of AKI, a logistic regression of AKI based on the preoperative metabolites and 24 h change was used. The values were log-transformed and normalized by the mean and standard deviation of the preoperative values. The distribution of P values was tested for uniformity using the Kolmogorov–Smirnov test.

Continuous variables are presented as median with interquartile range and were analyzed using Wilcoxon matched-pairs signed rank test for paired samples and Wilcoxon rank sum test for non-paired samples, adjusted to FDR. A pairwise comparison was performed between preoperative and postoperative samples. Enrichment and pathway metabolomic analysis were performed using the MetaboAnalyst 5.0 tool [15].

Results

Postoperative metabolic profile

The study included 53 patients: 33 underwent cardiac surgery with CPB, 10 underwent cardiac surgery without CPB, and 10 had major vascular surgery. Patients' demographics and baseline characteristics are presented in Table 1.

Untargeted metabolomics data for the complete population recorded up to 8,668 metabolic features for each serum sample collected before, and at 6 h and 24 h after surgery (Fig. 1A). Multivariate analysis, including pattern recognition tools such as principal component analysis (PCA) and linear discriminant analysis (LDA), showed a temporal dynamic with distinct metabolic profile changes across the three-time points.

For each group of patients, we investigated the time-dependent changes for each metabolic feature (Fig. 1A). Among patients who underwent cardiac surgery with CPB (CPB group), we identified 772 significantly altered features. Enrichment analysis identified five significant classes ($P < 0.05$), with glycine and serine metabolism

Table 1 Demographic data

Variable	All patients	CPB group	No CPB group	VascSurg group	*P* value
N	53	33	10	10	
Age, years, median (IQR)	70 (65.5–75)	74 (68.5–76)	67 (59.3–68.5)	66.5 (64.8–78.5)	0.025
Male sex, *n* (%)	33 (62.3)	16 (45.59)	8 (80)	9 (90)	0.026
BMI, kg/m^2, median (IQR)	27.8 (24.9–29.6)	27.8 (24.7–29.9)	27.7 (26.7–29.1)	27.4 (25.5–30.7)	0.97
Hypertension, *n* (%)	47 (88.7)	28 (84.8)	9 (90)	10 (100)	0.59
Diabetes mellitus, *n* (%)	20 (37.7)	11 (33.3)	5 (50)	4 (40)	0.63
Dyslipidemia, *n* (%)	31 (58.5)	14 (42.4)	9 (90)	8 (80)	0.009
Obesity, *n* (%)	11 (20.8)	7 (21.2)	2 (20)	2 (20)	0.99
Chronic kidney disease, *n* (%)	2 (3.8)	0 (0)	1 (10)	1 (10)	0.18
Cerebrovascular disease, *n* (%)	4 (7.5)	0 (0)	2 (20)	2 (20)	0.03
Chronic lung disease, *n* (%)	8 (15.1)	6 (18.2)	1 (10)	1 (10)	0.72
Aortic stenosis, *n* (%)	33 (62.3)	33 (100)	0 (0)	0 (0)	< 0.0001
Coronary artery disease, *n* (%)	10 (18.9)	0 (0)	10 (100)	0 (0)	< 0.0001
Peripheral vascular disease, *n* (%)	4 (7.5)	0 (0)	0 (0)	4 (40)	< 0.0001
Abdominal aortic aneurysm, *n* (%)	6 (11.3)	0 (0)	0 (0)	6 (60)	< 0.0001
Preoperative medical therapy, *n* (%)					
• Beta-blockers	17 (32.1)	10 (30.3)	5 (50)	2 (20)	0.33
• ARB or ACE inhibitor	31 (58.5)	16 (48.5)	9 (90)	6 (60)	0.07
• Statin	31 (58.5)	16 (48.5)	8 (80)	7 (70)	0.15
• Antiplatelet	26 (49.1)	14 (42.4)	6 (60)	6 (60)	0.46
• Diuretics	18 (33.9)	12 (36.4)	3 (30)	3 (30)	0.89
• Anticoagulant	2 (3.8)	1 (3)	0 (0)	1 (10)	–
Timing, *n* (%)					–
– Elective	53 (100)	33 (100)	10 (100)	10 (100)	
– Urgent	0 (0)	0 (0)	0 (0)	0 (0)	

BMI body mass index, *CPB* patients submitted to cardiac surgery with cardiopulmonary bypass, *No CPB: CV* patients submitted to cardiac surgery without cardiopulmonary bypass, *IQR* interquartile range, *VascSurg* patients submitted to major vascular surgery

being the most represented, followed by homocysteine degradation, methionine metabolism, the urea cycle, and ammonia recycling (Fig. 1B). Notably, all significant classes are related to protein metabolism. Among the 25 most represented classes, 16 were related to protein metabolism, 7 to lipid metabolism, and 2 to glucose metabolism (Fig. 1B). Pathway analysis identified 11 significant pathways ($P < 0.05$), with glycine, serine, and threonine metabolism being the most significant, followed by several other pathways related to protein metabolism (Fig. 1B).

In patients who underwent cardiac surgery without CPB (No CPB group), we identified 125 significantly altered metabolites. Enrichment analysis identified three statistically significant classes ($P < 0.05$): catecholamine biosynthesis, arginine and proline metabolism, and glycine and serine metabolism (Fig. 1C). Pathway analysis identified two significant pathways: arginine and proline metabolism, and vitamin B6 metabolism (Fig. 1C).

In patients who underwent major vascular surgery (VascSurg group), the number of significantly altered metabolites identified up to 24 h after surgery was markedly lower (63 metabolites). The reduced number of metabolites generated only two classes in the enrichment analysis, neither of which was statistically significant, and no pathways were generated in the pathway analysis (Fig. 1D).

Comparison of the metabolic profile between types of major cardiovascular surgery

To assess the impact of metabolic changes induced by each type of major cardiovascular surgery, we conducted PCA analysis using the log fold changes of metabolites between time points (Fig. 2A). Although the three groups share some similarities, the profiles of the No CPB and VascSurg groups were more similar, while the CPB presents a distinct and separated profile (Fig. 2A). The No CPB group exhibits an intermediate pattern between the other two groups.

We then evaluated the number of metabolites with significant changes and a peak intensity variation above 50%, common to all datasets. Between the samples

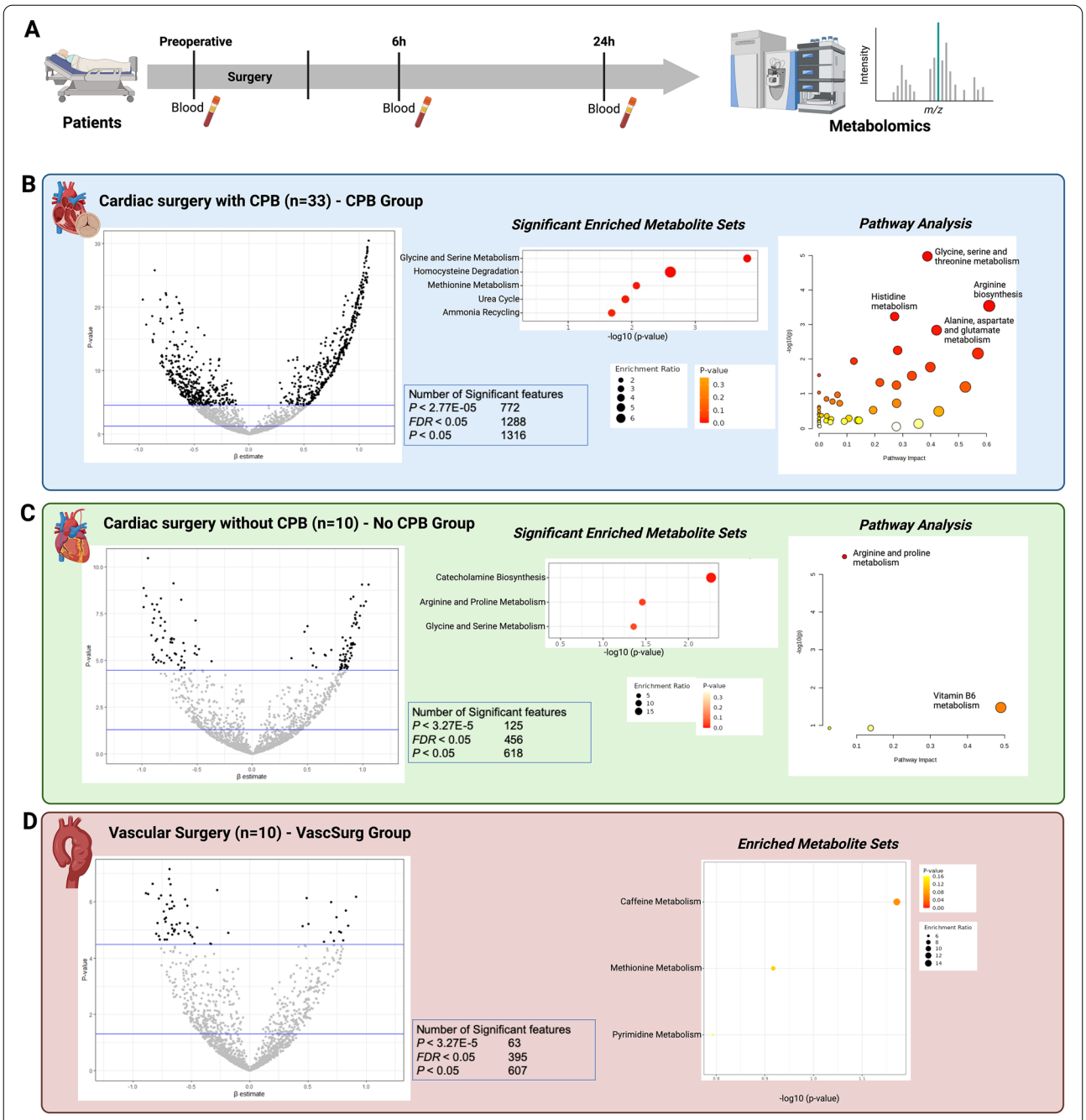


Fig. 1 **A** Schematic of study design. Using a linear mixed-effect model with Bonferroni correction, we investigated the time-dependent change up to 24 h of each feature in patients undergoing, **B** cardiac surgery with cardiopulmonary bypass (CPB) ($n = 33$), **C** cardiac surgery without CPB ($n = 10$) and **D** major vascular surgery ($n = 10$). We used the identified metabolites to perform enrichment and pathway analyses and we show the statistically significant classes and pathways in the central and right panels, respectively, of (**B**) and (**C**). In (**D**), no statistically significant classes were identified in the enrichment analysis, and therefore no pathway analysis was generated with the metabolite set

collected preoperatively and 6 h post-surgery, almost 300 metabolites showed significant differences, with only 167 metabolites displaying similar changes across all 3 groups (Fig. 2B). Specific changes included 50 metabolites exclusively to CPB group, 52 shared by CPB and No CPB groups, and 29 by CPB and VascvSurg groups (Fig. 2B). Using the same strategy, we identified changes in the metabolites between the samples collected preoperatively and 24 h post-surgery, as well as between 6 and 24 h post-surgery. We identified 170 metabolites

with significant changes across all 3 groups, with 45 metabolites altered exclusively in the CPB group, 70 in both the CPB and No CPB groups, and 23 in the CPB and VascSurg groups (Fig. 2B). Between 6 and 24 h post-surgery, 128 metabolites showed significant changes in all groups, with 25 metabolites altered exclusively in the CPB group, 46 in both the No CPB and CPB groups and 13 in both the VascSurg and CPB groups (Fig. 2B).

Finally, we used log fold changes of the entire set of metabolites as input for propagation across the metabolic network, comparing pathways across the three groups (Fig. 2C). Comparing the impact of the pathways across the three groups, CPB group showed a strong association with protein metabolism, with significantly elevated propagation scores for pathways such as protein digestion and absorption, glycine, serine and threonine metabolism, D-amino acid metabolism, biosynthesis of cofactors, biosynthesis of amino acids and 2-oxocarboxylic acid metabolism (Fig. 2C). This pattern persisted when comparing preoperative metabolites to those 24 h post-surgery (Fig. 2C), with a significant overrepresentation of protein-related pathways and distinct propagation scores in the CPB group. Both at 6 h and 24 h after surgery, the SurgVasc and No CPB groups had similar propagation scores, which were considerably lower than those observed for the CPB group. During the transition from 6 to 24 h after surgery, the CPB group was strongly associated with increased protein metabolism, while the No CPB group presented intermediate scores compared to the VascSurg group, which had the lowest scores for the identified pathways (Fig. 2C).

Protein metabolism and the occurrence of postoperative acute kidney injury

We examined how increased protein metabolism in patients undergoing cardiac surgery with CPB (CPB group) is related to postoperative AKI, recognizing the kidney's essential role in managing the amino acid reserve by absorbing or releasing amino acids [16]. In the CPB group, 14 patients (42.4%) developed postoperative AKI, with the majority (11 patients, 33.3%) having stage 1 and 3 patients (9.1%) having stage 2 AKI.

First, by characterizing preoperative metabolites in CPB patients who developed postoperative AKI, we identified several classes related to catabolism. These included the transfer of acetyl groups into mitochondria, valine, leucine, and isoleucine degradation, ketone body metabolism, threonine and 2-oxobutanoate degradation, propanoate metabolism, pyruvate metabolism and gluconeogenesis (Fig. 3A). Similar pathways were identified with pathways analysis (Fig. 3B).

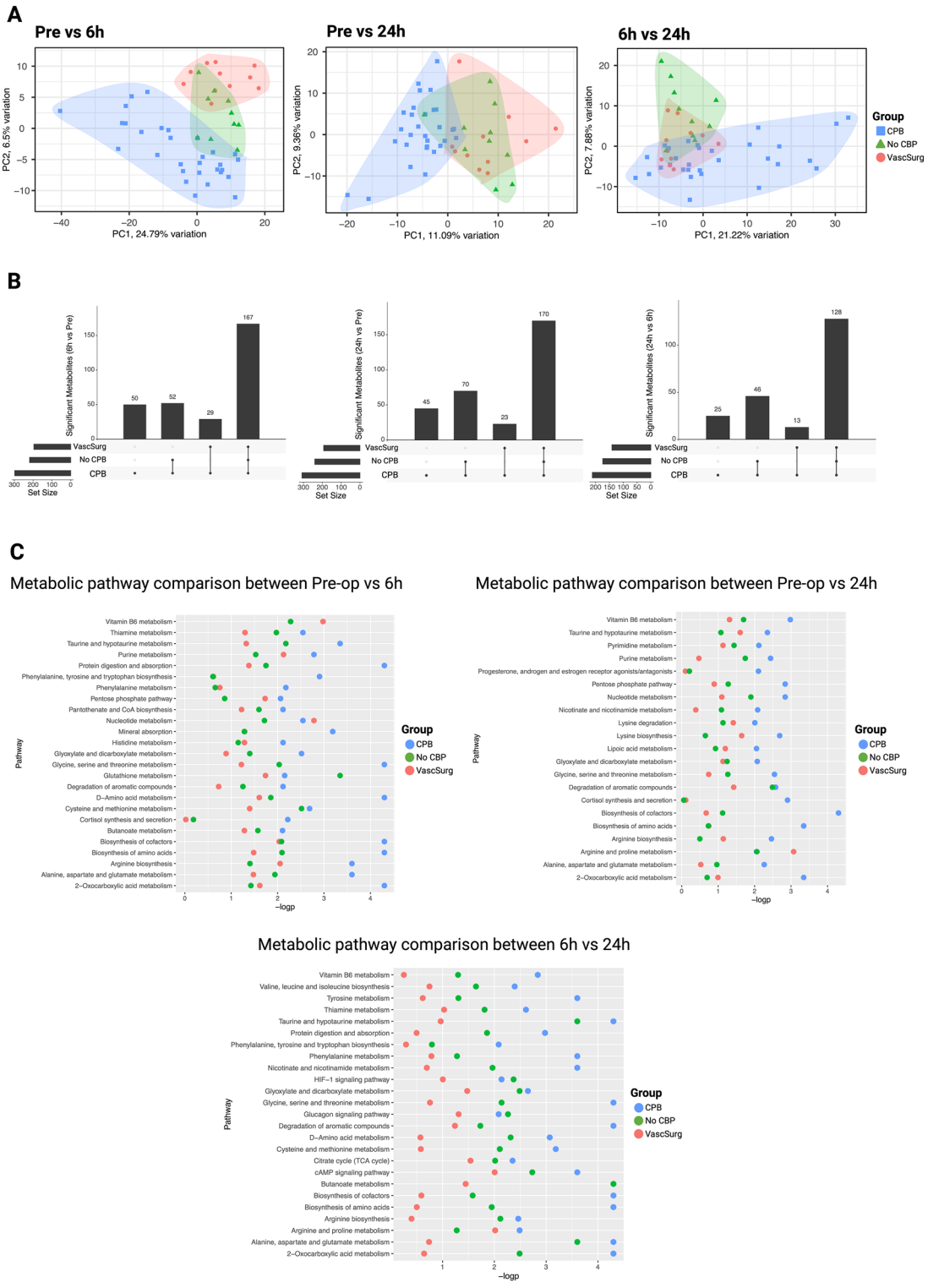
Then we investigated metabolites that changed significantly from preoperative to 24 h after surgery and were linked to postoperative AKI, identifying 101 such metabolites (Supplementary Table 1). Enrichment and pathway analyses revealed the citric acid cycle (TCA cycle) as the most represented and significantly affected pathway related to postoperative AKI (Fig. 3C). Preoperative citric acid levels were significantly higher in patients who developed AKI (3.49×10^8 vs 2.66×10^8 ion-count, $P=0.010$), and these levels further increased 24 h postoperative (2.64×10^8 vs 1.76×10^8 ion-count, $P=0.0008$). Significant increases in alpha-ketoglutaric acid, fumarate, and malate were also observed 24 h postoperative (Fig. 4). Other relevant classes included homocysteine degradation, the malate-aspartate shuttle, lysine degradation, the Warburg effect, and aspartate metabolism.

Interestingly, among the 101 significant metabolites, 7 were acylcarnitines (Fig. 3C). Levels of *O*-3-methylglutarylcarnitine were significantly higher in AKI patients preoperatively (1.11×10^7 vs 0.72×10^7 ion-count, $P=0.015$), at 6 h (2.77×10^7 vs 1.52×10^7 ion-count, $P=0.0113$) and 24 h post-surgery (3.15×10^7 vs 2×10^7 ion-count, $P=0.0061$). At 24 h post-surgery, we also noted increased levels of decanoylcarnitine,3-hydroxyoctanoylcarnitine, 9-decenoylcarnitine, trans-2-dodecenoylcarnitine, 3,5-tetradecadiencarnitine and 3-hydroxy-cis-5-tetradecenoylcarnitine.

Preoperative levels of citric acid and *O*-3-methylglutarylcarnitine were notably higher in patients who developed postoperative AKI. Specifically, preoperative *O*-3-methylglutarylcarnitine was significantly associated with postoperative AKI ($P=0.035$), showing an odds ratio (OR) of 9.99 (95% CI 1.18–84.67) for a 0.5×10^7 increase. It also served as a good predictor of postoperative AKI,

(See figure on next page.)

Fig. 2 **A** Principal component analysis was performed using the log fold change in metabolites intensities, comparing the three groups (CPB, No CPB, VascSurg) for preoperative vs 6 h (left), preoperative vs 24 h (center) and 6 h vs 24 h (right). **B** Considering common metabolites across the three groups, a linear generalized model was used to evaluate the variation in metabolites among all groups, again comparing preoperative vs 6 h (left), preoperative vs 24 h (center) and 6 h vs 24 h (right). Each bar corresponds to the number of metabolites changed. **C** Comparison of the metabolic network across the three groups was conducted using the log fold changes of the entire set of metabolites as input for propagation. Metabolites from the three groups (CPB, No CPB, VascSurg) were compared at preoperative vs 6 h metabolites (top), preoperative vs 24 h (middle), and 6 h vs 24 h (bottom). CPB: cardiac surgery with CPB group; No CPB: cardiac surgery without CPB group; VascSurg: major vascular surgery group



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with an area under the curve of 90.7% (Fig. 3D). The predicted probability of postoperative AKI increased with higher preoperative *O*-3-methylglutarylcarnitine levels, with a 50% risk at around 0.9×10^7, reaching 100% risk at approximately 1.6×10^7 ion-count (Fig. 3E).

Discussion

Major surgery is known to cause significant organ dysfunction, but the underlying molecular mechanisms remain undetermined. Here, we address these gaps using an untargeted metabolomic approach to explore the effects and clinical implications of surgical-stress-induced metabolic reprogramming.

The first key finding of this study is a distinct and specific metabolic signature associated with cardiac surgery using CPB, characterized by an overrepresentation of protein metabolism. Although cardiac surgery without CPB and major vascular surgery also affect postoperative metabolism, the impact is considerably less pronounced.

In patients undergoing cardiac surgery with CPB, the most increased metabolic pathways include glycine, serine, and threonine metabolism, along with arginine biosynthesis. These upregulations likely result from immune cell activation induced by the characteristic postoperative SIRS [3]. Serine, a non-essential amino acid, is crucial for maintaining metabolic homeostasis under pathological and stress conditions [18], supporting IL-1β production by macrophages and promoting immune cells proliferation [19, 20]. In addition, serine is important for synthesizing phospholipids and sphingolipids, and therefore responsible for lipid metabolism homeostasis [18, 21]. This metabolic signature in cardiac surgery patients may represent a compensatory mechanism to restore lipid balance amid the increase in postoperative protein metabolism, suggesting potential therapeutic uses for serine.

Arginine, another non-essential amino acid, is a critical regulator of immune responses, especially in macrophages, dendritic cells, and T cells. It stimulates the release of anabolic hormones like adrenal catecholamines [22–24], essential for cardiovascular hemodynamics and wound healing [25]. Interestingly, arginine supplementation, studied since the 1980s as part of immune-enhancing diets, has shown benefits in reducing infections and enhancing immunity in high-risk surgical, trauma, and septic patients [26–28]. In addition, systemic activation of branched-chain amino acids (BCAA) is cardioprotective after myocardial ischemia [29]. The observed metabolic signature in this study may reflect a protective host response to surgical stress.

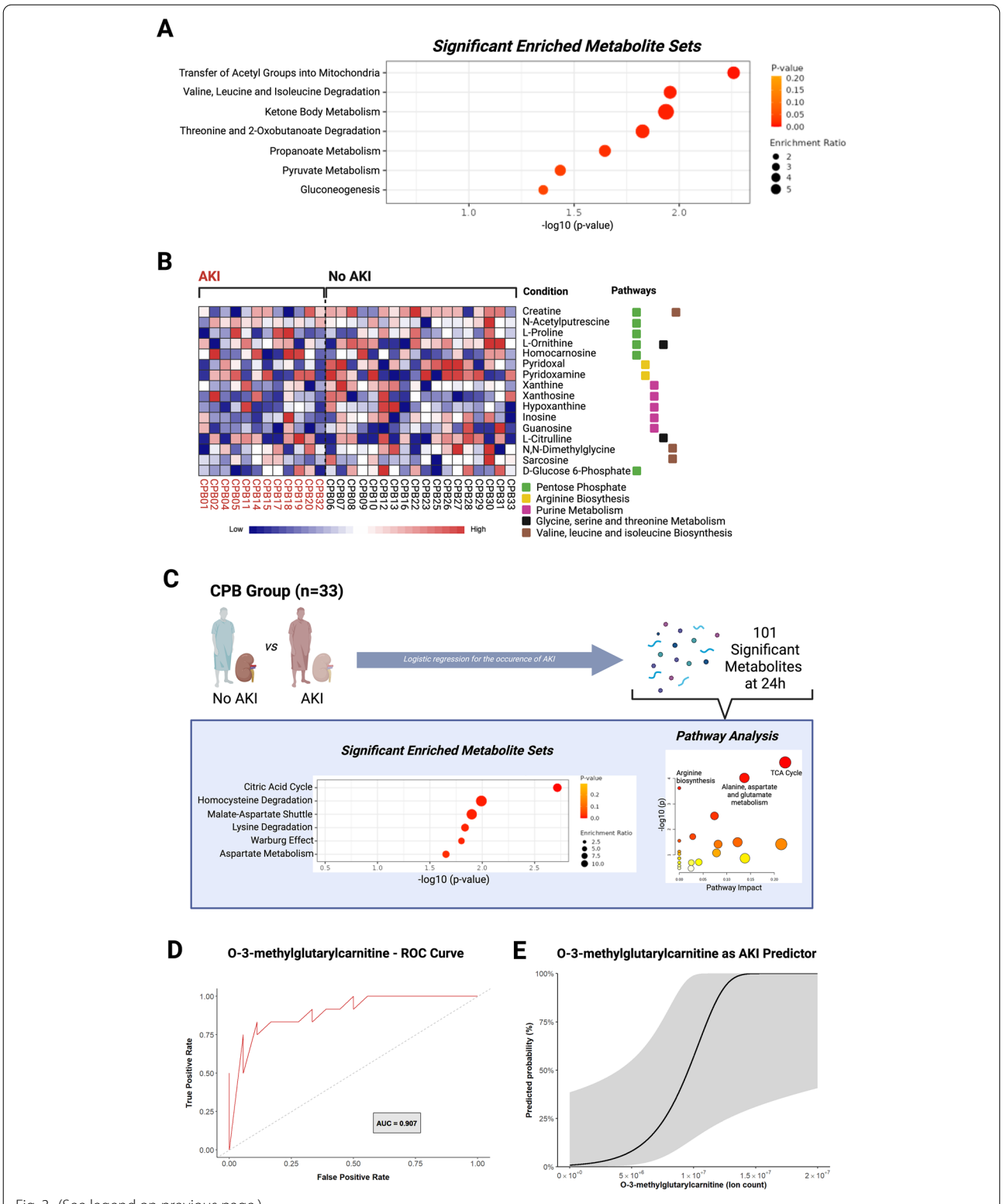
The second key finding of our study is the association between increased protein catabolism and postoperative AKI [23]. We consider this a very important finding, given that AKI is a common complication of cardiac surgery and a major prognostic factor for these types of interventions [30–32]. A central factor for this association is decreased glomerular filtration rate (GFR) in response to renal hypoperfusion resulting from CPB [30–32]. The kidney is crucial in regulating circulating amino acids and maintaining amino acid pool homeostasis [16], synthesizing and releasing amino acids like serine, tyrosine, arginine, threonine, lysine, and leucine [33]. Most of these amino acids were significantly impacted by cardiac surgery with CPB. In addition, increased plasma proteins modulate kidney hemodynamics, boosting renal blood flow and glomerular filtration rate.

We also observed that patients who developed postoperative AKI showed significant increases in three pathways: (1) transfer of acetyl groups into mitochondria, (2) valine, leucine, and isoleucine degradation, and (3) ketone body metabolism. The occurrence of AKI was associated with increased preoperative levels of citric acid and O-3-methylglutarylcarnitine, along with elevated TCA cycle metabolites and acylcarnitines after surgery. In addition, systemic activation of BCAA catabolism lowers blood pressure and vascular resistance [29], potentially inducing or worsening renal ischemia, especially in patients with pre-existing kidney disease or in the context of a major cardiovascular surgery.

The identification of distinct concentrations of acylcarnitines in patients with postoperative AKI reveal, for the first time, their role in the heart-kidney axis disease mechanism. Acylcarnitines are fatty acid metabolites used as markers of energy metabolism, that transport

(See figure on next page.)

Fig. 3 **A** Preoperative metabolic profile of CPB patients (cardiac surgery with CPB group) who developed postoperative acute kidney injury (AKI) at 24 h. Enrichment analysis identified seven statistically significant classes, including the transfer of acetyl groups into mitochondria and several classes related to protein catabolism. **B** Pathway analysis identified five significant pathways in patients who developed postoperative AKI compared with patients without postoperative AKI (No AKI group). The different pathways are coded with different colors on the right. **C** A logistic regression for the occurrence of acute kidney injury (AKI) within 24 h identified 101 significantly changed metabolites. Enrichment (left) and pathway (right) analyses revealed the citric acid (TCA) cycle as the most significant pathway, along with other classes related to protein catabolism. **D** The receiver operator characteristic (ROC) curve demonstrated the predictive value of 3-methylglutarylacylcarnitine for postoperative AKI, with an area under the ROC curve (AUC) of 0.907. **E** The predicted probability of postoperative AKI increased with higher preoperative levels of *O*-3-methylglutarylcarnitine.The grey area corresponds to the 95% confidence interval



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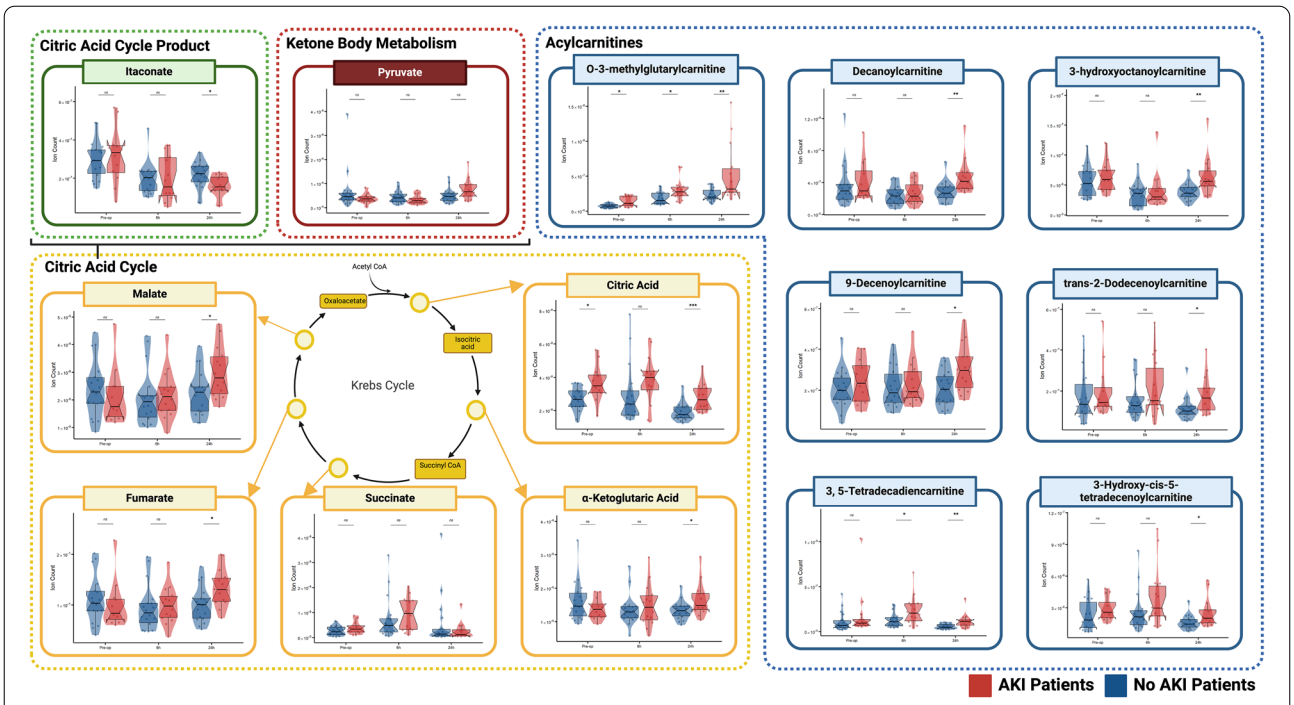

Fig. 4 In CPB patients (cardiac surgery with CPB group) with postoperative acute kidney injury (AKI), the citric acid cycle is disrupted, showing significantly increased levels of citric acid, and slight increases in alpha-ketoglutaric acid, fumarate and malate. Seven acylcarnitines were identified among the significant metabolites associated with the occurrence of postoperative AKI, all showing higher levels in patients with AKI. In addition, itaconate levels were significantly decreased in patients with postoperative AKI. Red refers to AKI patients; blue refers to no AKI patients. Significance levels are denoted as follows: Ns: non-significant; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$

acyl groups from the cytosol into the mitochondrial matrix for energy production [34]. Increased acylcarnitine production represents a critical mediator of metabolic flexibility, allowing mitochondria to use appropriate substrates based on physiologic and nutritional needs [35]. Cardiometabolic diseases are often linked with metabolic inflexibility and mitochondrial dysfunction, impaired fuel switching, and energy dysregulation [35]. Abnormal acylcarnitine levels reflect intracellular levels and the regulation of acetyl-CoA and are associated with various cardiovascular conditions, such as chronic heart failure, diastolic heart failure, coronary artery disease, or cardiac arrhythmias [36–39]. Plasma acylcarnitines reflect cardiac acylcarnitine content and subsequent mitochondrial dysfunction [34, 40, 41], with multiple studies in rodents, pigs, and humans confirming that long-chain acylcarnitines from skeletal muscle do not significantly affect plasma levels [40].

Cardiac surgery with CPB leads to myocardial ischemia, inducing acylcarnitines accumulation [34] that disrupts energy metabolism in the myocardium during ischemia–reperfusion by inhibiting oxidative phosphorylation (OXPHOS) [42, 43]. Protein catabolism, especially from BCAA such as valine, leucine, and isoleucine, is a major source of short- to medium-length acylcarnitines. Our data suggest that myocardial ischemia associated with cardiac surgery, combined with increased protein catabolism, contribute to plasmatic acylcarnitines accumulation. This disrupts OXPHOS, accumulating citric acid and altering TCA cycle activity. Surgical stress, rather than cardiac dysfunction alone, seems to drive these changes since modifications in cardiac metabolism and energy substrates induced by cardiac dysfunction do not affect acylcarnitine production [34]. Increased TCA cycle metabolites in both plasma and kidney tissue during renal ischemia–reperfusion have been previously described, suggesting that citrate accumulation may indicate a block in TCA cycle progression and metabolism reduction [44].

Furthermore, protein breakdown during extreme fasting can also contribute to TCA cycle substrates, supporting our observations [44]. Another observation from our data supporting this hypothesis is that itaconate levels were significantly increased at 24 h in patients without AKI. Itaconate is an immunometabolite that slows TCA cycle metabolism and is protective in the host response to ischemia and reperfusion [45]. Specifically in the kidney, its anti-inflammatory role in inducing immune tolerance and controlling innate immunity has been shown to be protective against AKI [45, 46].

Intriguingly, acylcarnitines are closed linked to inflammation, activating proinflammatory pathways [47], modulating inflammatory cells activity and the production of cytokines [47, 48]. Elevated plasmatic acylcarnitines have been found in sepsis non-survivors [49]. Evidence from murine models suggests that the anti-inflammatory macrophage response is more robustly supported by mitochondrial OXPHOS than by fatty acid oxidation. The availability of acylcarnitines determines the energy metabolic pattern in immune cells, emphasizing the role of immunometabolism in disease mechanisms [34, 50].

While our study combines clinical data with circulating metabolite levels to characterize metabolic changes from major cardiovascular surgery, it is limited by the still small, predominantly male patient cohort, which makes the generalization of these findings to other groups unclear. Moreover, chronic kidney disease was not an exclusion criterion, although no patients in the CPB group presented it, and the logistic regression of AKI was adjusted to sex, age, preoperative glomerular filtration rate (GFR) and creatinine levels, and GFR and creatinine levels at 24 h to reduce possible implication of different renal function levels on metabolic signatures.

Our study has important strengths and clinical implications. First, we detailed metabolic perturbations induced by surgical stress in major cardiovascular surgeries, pointing to specific metabolic patterns associated with postoperative AKI. Prominently among them is a striking increase in protein metabolism. Interestingly, most protein metabolism pathways identified in our data are related to amino acids the kidney regulates. Notably, our findings are well aligned with the recent large clinical trial involving 3511 patients, demonstrating that the infusion of amino acids significantly reduced the occurrence of AKI in adults undergoing major cardiac surgery [52]. This important result is consistent with previous animal studies and prior small randomized clinical trials, that have demonstrated that a short-term amino acid infusion increases GFR [53–59]. Second, we identified *O*-3-methylglutarylcarnitine as a potential predictor of postoperative AKI.

These discoveries may lead to novel therapeutic interventions, including those based on new drug targets, and inform the development of nutritional strategies to improve outcomes. For example, drugs that target acylcarnitine synthesis and transport or preoperative nutritional interventions that modulate their plasma concentrations should be explored to improve the outcome of major cardiac surgeries. This possibility is illustrated by a potent inhibitor of L-carnitine biosynthesis and transport that has recently been shown to decrease the accumulation of inflammatory cells and the progression of atherosclerosis [60]. An additional possibility is the identification of the most effective amino acids that improve post-major heart surgery outcomes and their mechanisms of action in the kidney. This strategy can potentially uncover novel, effective targets to transform the natural history of these surgical procedures. In conclusion, our findings suggest opportunities for new cardiometabolic interventions to modulate cardiac and host metabolism, improving morbidity in major cardiovascular surgeries.

Supplementary Information
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Author contributions
TRV: investigation, methodology, project administration, validation, writing—original draft, writing—review and editing; FP: validation, data curation, data analysis; RF: conceptualization, investigation, methodology, project administration, supervision, writing—review and editing; RMP: conceptualization, methodology, validation, data curation, data analysis, writing—review and editing; AD: investigation, methodology, project administration; MH: data curation, data analysis; KW: investigation, data curation, validation, writing—review and editing; DP: investigation, data curation, validation, writing—review and editing; TP: data curation, data analysis; NCG, ANC, IS, RGM, BD, AM, AN, RWS, TK, LMP, FP: validation, writing—review and editing; LFM: conceptualization, investigation, methodology, project administration, supervision, validation, data curation, writing—original draft, writing—review and editing.

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Data availability
Research data will be made available upon reasonable request to the corresponding author.

Declarations

Conflicts of interest
The authors declare that they have no conflict of interest.

Ethical approval
This study was approved by the review board of Hospital de Santa Maria, Unidade Local de Saúde Santa Maria (Ref. N.º 23/18).

Institutional review board
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