

Metabolomic Signatures Influenced by Different Cardioplegic Solutions in Cardiac Surgery

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Abstract

Introduction: Cardioplegia is crucial for myocardial protection during cardiac surgery. However, the impact of different cardioplegia formulations on patients' metabolomic responses has not been explored. The objective is to identify unique metabolomic signatures associated with each cardioplegia type and evaluate their potential correlations with clinical outcomes. **Methods:** A prospective cohort study was conducted from January 2023 to June 2024, involving 105 samples from 21 patients undergoing isolated coronary artery bypass graft with modified del Nido, Custodiol®, or St. Thomas cardioplegia. Pre- and postoperative plasma samples were collected for analysis of cardiac enzyme levels and proton nuclear magnetic resonance metabolomic profiling. **Results:** No significant differences in clinical outcomes or cardiac enzyme levels were observed between the groups. However, distinct pre- and postoperative metabolomic profiles were identified. The Custodiol group exhibited significant postoperative metabolomic changes within the first 2 days, with nine differentially expressed metabolites (DEMs). In contrast, modified del Nido cardioplegia affected three DEMs on postoperative day 0, whereas St. Thomas showed no alteration in the postoperative metabolomic profile. These DEMs were associated with protein and energy production, oxidative stress, and inflammatory responses. Key differential metabolites between the groups included histidine and alanine, which notably impacted the histidine metabolism pathway. **Conclusion:** The findings demonstrate that distinct cardioplegia solutions result in differential postoperative metabolomic alterations, with Custodiol exhibiting the most pronounced shifts, whereas St. Thomas cardioplegia showed no significant postoperative metabolic changes.

Keywords: Coronary artery bypass graft, cardioplegia, differentially expressed metabolites, metabolomics profiles

INTRODUCTION

Cardioplegia is essential in cardiac surgery, providing myocardial protection during surgical procedures by inducing cardiac arrest.^[1] It functions through mechanisms such as enhancing oxygen delivery, buffering capacity, capillary flow optimization, free radical management, and oncotic pressure maintenance.^[2] While numerous clinical studies have assessed the effects of various cardioplegia solutions, their impact on patients' metabolic profiles has never been explored. Metabolomic studies, which analyze metabolites responsive to various biological conditions or interventions, offer valuable insights into physiological changes.^[3] Our study investigates the metabolomic profiles of coronary artery bypass graft (CABG) patients receiving modified del Nido, Custodiol, and St. Thomas cardioplegia formulations which are commonly used in clinical practice. We use

proton nuclear magnetic resonance (¹H NMR) techniques to examine how these solutions affect postoperative metabolomic responses. This comparative analysis aims to enhance our understanding of pathophysiology and facilitate the optimization of cardioplegic strategies and perioperative management.

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METHODS

Study design and population

From January 2023 to June 2024, we conducted a prospective cohort study at Srinagarind Hospital and Queen Sirikit Heart Center of the Northeast. The study was approved by the IRB and Ethics Committee of Khon Kaen University's Faculty of Medicine. Written informed consent was obtained from all patients. A total of 105 blood samples were collected from 21 patients scheduled for surgery, divided into three groups based on the type of cardioplegia administered, which was determined by the surgeon's preference. The sample size was calculated based on a previous study^[4] to detect significant metabolite differences between the groups. Inclusion criteria encompassed patients aged >18 years undergoing isolated CABG, whereas exclusion criteria included poorly controlled diabetes (HbA1c >8 or insulin use), serum creatinine levels exceeding 2 mg/dL, jaundice, use of immunosuppressive drugs, immune diseases, cancer, or the need for emergency surgery [Figure 1].

Operative procedure and sample collection

All patients received standardized general anesthesia. Uniform procedures were performed by experienced CABG surgeons through median sternotomy. Cardiopulmonary bypass was initiated by cannulating the ascending aorta and right atrium. Cardiac arrest was induced using antegrade cardioplegia through the aortic root, with the choice of solution determined

by the surgeon's preference. Modified del Nido cardioplegia prepared in-hospital with lactated Ringer's solution substituting Plasma-Lyte A while retaining the original formula for other components or Custodiol® HTK Solution. Both were administered as a single dose, whereas St. Thomas cardioplegia was given in repeated doses every 20 min. After the final distal anastomosis, the aortic cross-clamp was removed, and proximal anastomosis was performed with the heart beating.

Blood samples were collected preoperatively and on postoperative days 0–3. Two milliliters of blood were collected and stored in heparinized tubes (VACUETTE®, Austria), centrifuged at 9000 rpm for 15 min at 4°C, and the plasma was aliquoted and stored at –80°C for future use.

Sample preparation and proton nuclear magnetic resonance spectral data analysis

Plasma samples were analyzed at various time points based on cardioplegia type. A 400 µL plasma sample was mixed with 400 µL of 0.142 M phosphate buffer in D₂O (pH 7.4), containing 3 mM sodium azide and 0.08% trimethylsilylpropanoic acid (TSP). The mixture was vortexed, sonicated at 25°C for 15 min, and centrifuged at 13,000 × g at 4°C for 10 min. A 600 µL supernatant sample was transferred into a 5-mm NMR tube for ¹H NMR spectroscopy using a 400 MHz spectrometer (Bruker, USA), employing the Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence with water signal suppression over 64 scans. Data processing was

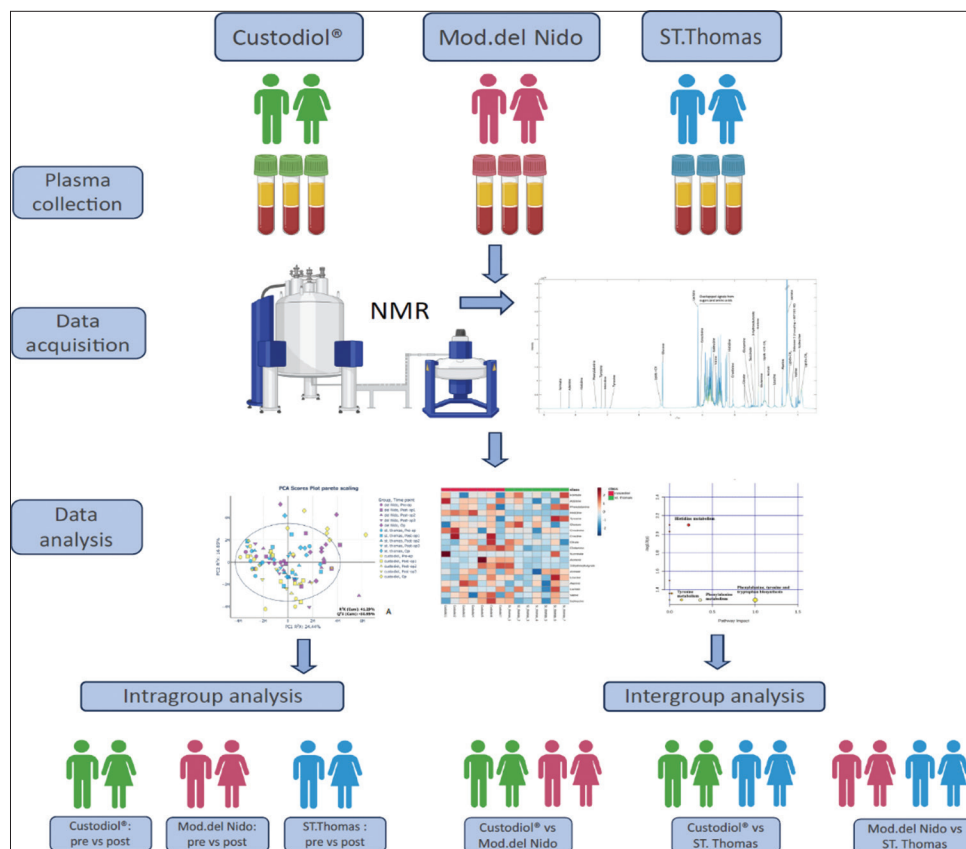


Figure 1: The metabolomics study flow. Pre: Presurgery, Post: Postsurgery. Created by BioRender.com.

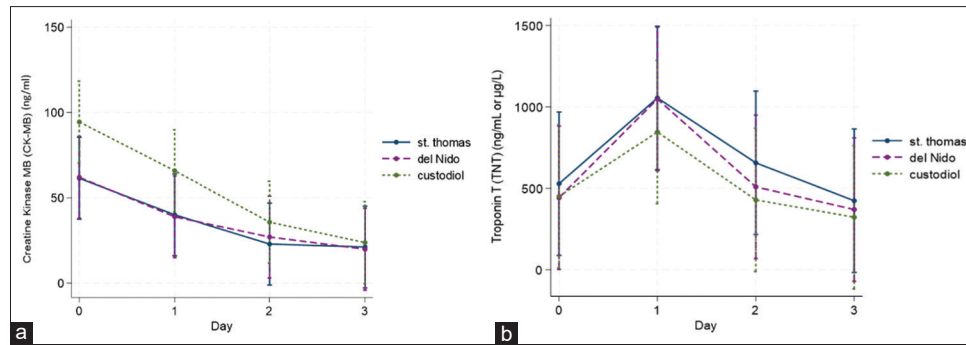


Figure 2: Linear graph predictions of CK-MB level (a) and Troponin T level (b) from the Generalized Estimating Equations model. Comparing three types of cardioplegia over four time points: Days 0, 1, 2, 3. POD: Postoperative day.

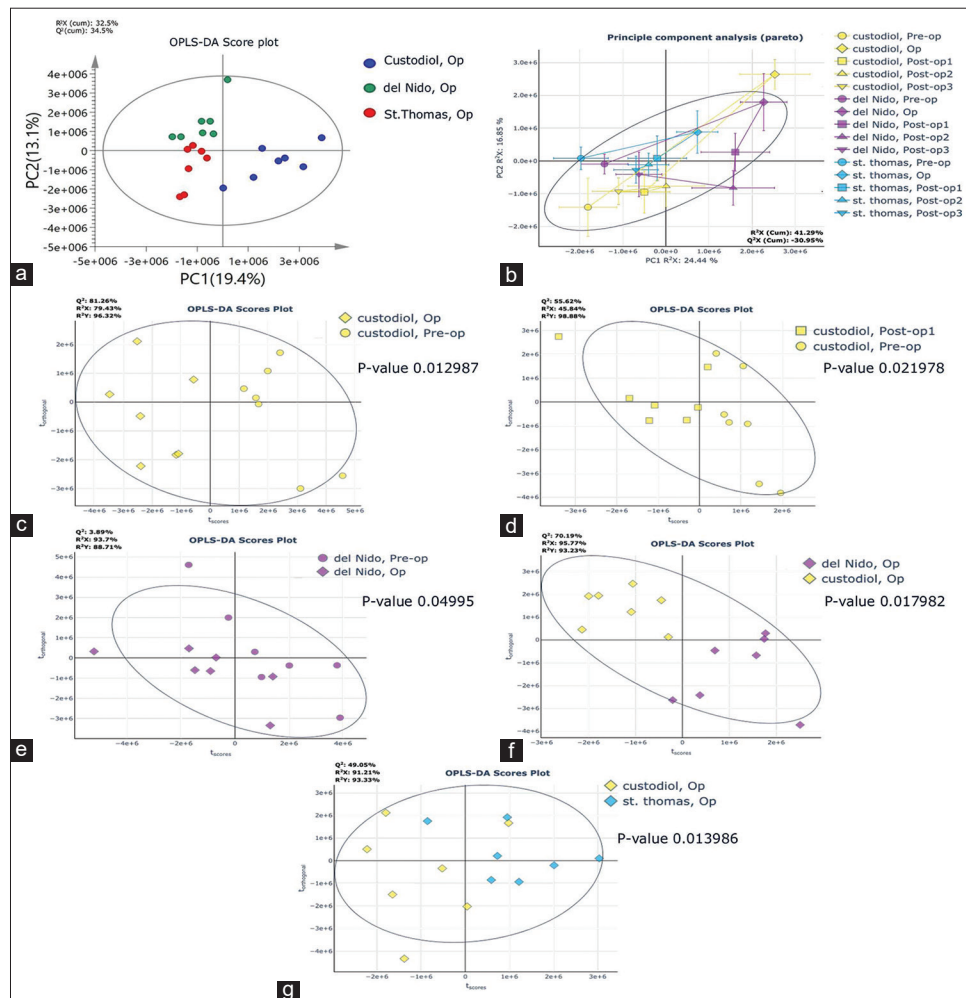


Figure 3: Metabolomic Analysis of Cardioplegia Solutions. (a) Orthogonal partial least squares discriminant analysis (OPLS-DA) comparing all cardioplegia types on postoperative day 0. (b) Time trajectory plot showing postoperative metabolic shifts over time for all cardioplegia types. (c and d) OPLS-DA for the Custodial group comparing preoperative and postoperative day 0, and day 1, respectively. (e) OPLS-DA for the modified del Nido group comparing preoperative and postoperative day 0. (f) OPLS-DA comparing Custodial and modified del Nido groups on postoperative day 0. (g) OPLS-DA comparing Custodial and St. Thomas groups on postoperative day 0. OPLS-DA: Orthogonal partial least squares discriminant analysis; OP: Operative day.

performed using Topspin software and in-house MATLAB scripts. Phase and baseline corrections were applied, and the TSP peak was set to 0 ppm. The water peak (δ 4.2– δ 5.2) was excluded after alignment. Statistical total correlation

spectroscopy (STOCSY) identified metabolites by generating pseudo-two-dimensional spectra, cross-referenced with the Human Metabolome Database (HMDB), ChenomxNMR Suite, and literature. Peak concentrations were determined by

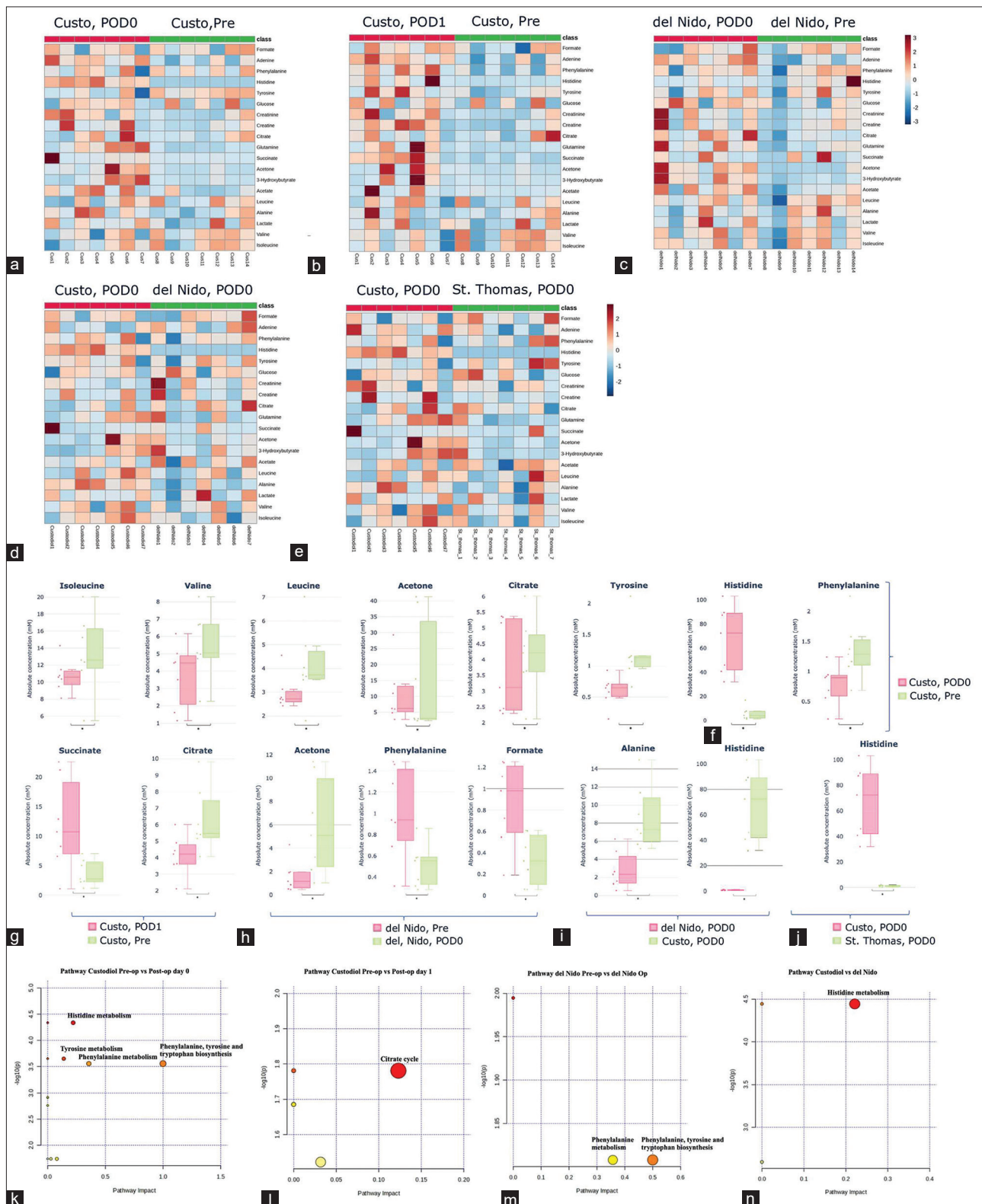


Figure 4: A comprehensive visual representation of the study findings utilizing various types of plots: Heatmap diagrams (a-e): Show relative abundance of differentially expressed metabolites (DEMs). Boxplots (f-j): Illustrate concentrations of significant DEMs in plasma across time points and groups. Pathway analysis summary (k-n): Summarize pathway analysis of differential metabolites, significant pathways with impact score > 0.1, false discovery rate < 0.25, and $P < 0.05$ or $-\log_{10}(p) > 1.3$. The comparison groups are as follows: Panels a and f and k: Custodial preoperative vs. postoperative day 0, Panels b and g and l: Custodial preoperative versus postoperative day 1, Panels c and h and i: modified del Nido preoperative versus postoperative day 0, Panels d and e and n: Custodial versus modified del Nido on postoperative day 0, Panels e and j: Custodial versus St. Thomas on postoperative day 0.

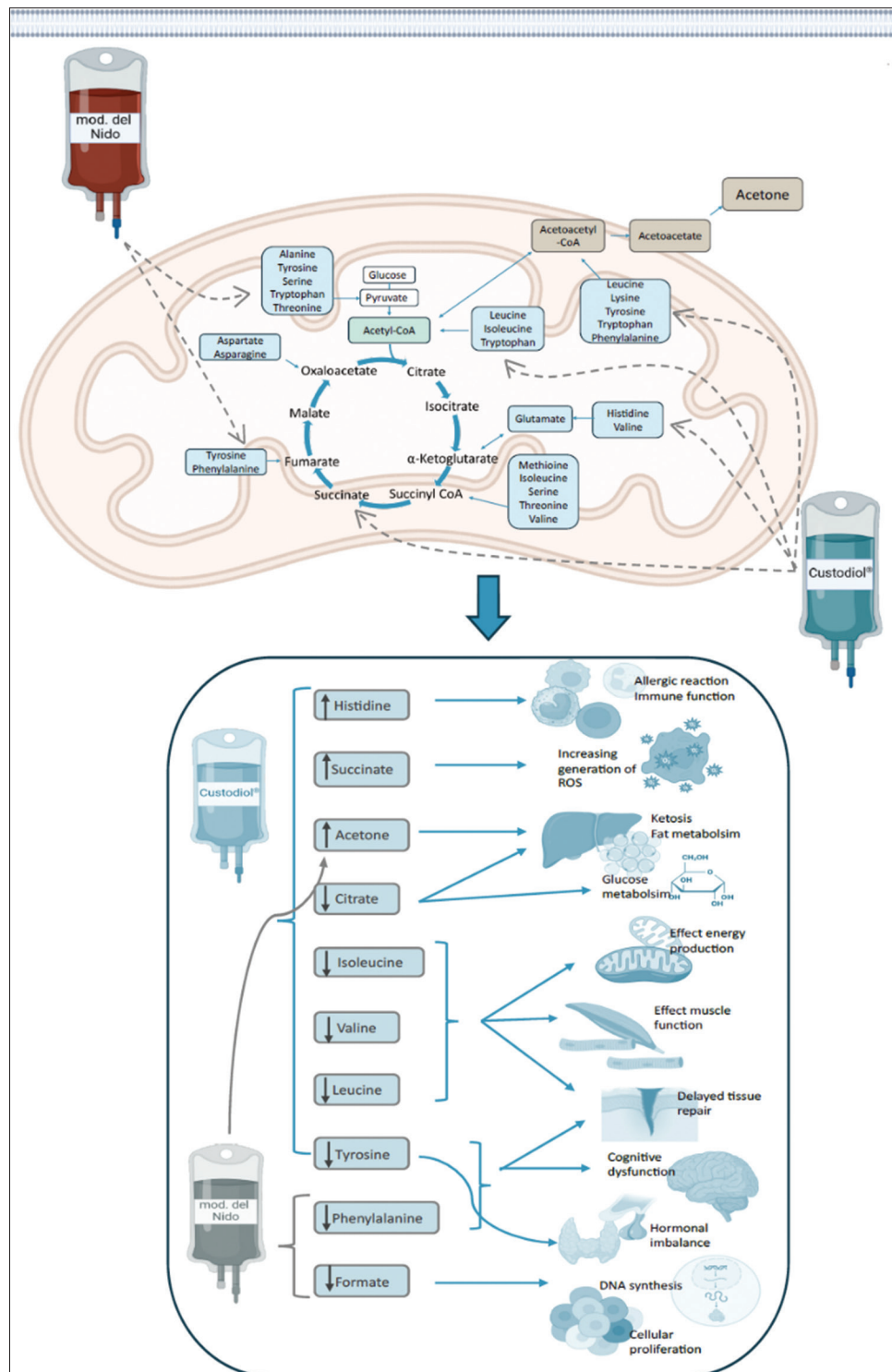


Figure 5: The significant metabolic pathways and differentially expressed metabolites affected by the type of cardioplegia, highlighting potential clinical implications of these metabolite perturbations. Created with BioRender.com.

comparing the integrals of the peaks of interest with the TSP internal control.

Clinical data analysis

Continuous data were assessed for normality using the Shapiro–Wilk test. Normally distributed data are presented

as mean and standard deviation (SD) and analyzed using One-way ANOVA. For nonnormally distributed data, the median and interquartile range (IQR) are reported, and the Kruskal–Wallis test is utilized for nonparametric comparison of medians across groups. Categorical data are presented as counts and percentages, with statistical analysis conducted

using Pearson's Chi-squared test or Fisher's exact test. For longitudinal data, such as cardiac enzyme levels, generalized estimating equations (GEEs) are employed to account for intragroup and intergroup correlations.

Univariate and multivariate statistical analysis of nuclear magnetic resonance spectral data

Data analysis, model creation, evaluation, and visualizations were conducted using SIMCA software 14, Python (version 3.12.1) with packages including pandas (version 1.1.3), NumPy (version 1.19.2), and the associated GitHub repository (<https://github.com/aeiwz/metbit>). Analysis was performed in Visual Studio Code. Model validity was assessed through cross-validation and permutation testing with significance set at $P < 0.05$. Orthogonal partial least squares discriminant analysis (OPLS-DA) was used, identifying significant variables based on a P (corr) cutoff of $|0.5|$ and Variable Importance in Projection (VIP) scores >2.0 . Absolute concentrations of differential metabolites were determined relative to TSP using the method described by Phetcharaburanin *et al.*^[5] The Mann–Whitney U test in SPSS version 25 was used for statistical analysis, with $P < 0.05$ considered significant. Heatmap analysis and metabolic pathway analysis were performed using MetaboAnalyst 6.0.^[6]

RESULTS

Patients

The demographic data in Table 1 show no significant differences between the groups, except for hemoglobin levels, which were significantly lower in the modified del Nido cardioplegia group.

Intraoperative data analysis revealed that the Custodiol group had more graft anastomoses, longer aortic cross-clamp times, and a greater volume of cardioplegia compared to the other groups. However, no significant differences were found in other intraoperative parameters [Table 2].

The postoperative analysis revealed no statistically significant differences among the groups in key outcomes, including postoperative complications and parameters such as creatinine levels, cardiac enzyme concentrations [Table 2], inotropic support requirements, and duration of ventilatory assistance [Supplementary Table 1]. Although the modified del Nido group exhibited longer average hospital stays, this difference did not reach statistical significance. Notably, there were no incidences of perioperative myocardial infarction or inhospital mortality. The Generalized Estimating Equation (GEE) analysis and the graphical trend of post operative cardiac enzyme level in Figure 2 also confirm the absence of group differences.

Metabolomics study results

Metabolic profiling

Metabolomic profiles were assessed using the OPLS-DA [Figure 3a], which clearly distinguished between the three cardioplegia types: Custodiol, modified del Nido, and St. Thomas, on postoperative day 0. The time trajectory plot [Figure 3b] illustrated that all cardioplegia types induced metabolic shifts postoperatively, with Custodiol associated with the most significant changes, and St. Thomas with the least. Heatmaps [Figure 4a-c] revealed variations in metabolite abundances in intragroup comparisons from preoperative to postoperative stages, while intergroup analyses [Figure 4d and e] delineated differences among the cardioplegia types.

Table 1: Demographic data of the patients

Parameter	Total (n=21)	Cardioplegia			P
		St. Thomas (n=7)	Del Nido (n=7)	Custodiol (n=7)	
Age (years), mean (SD)	63.71 (9)	67.43 (4.2)	65.43 (10.78)	58.29 (9.05)	0.134
Sex, male, n (%)	14 (66.67)	6 (85.71)	3 (42.86)	5 (50.00)	0.053
Height (cm), mean (SD)	161 (10.23)	162.71 (10.45)	155.14 (11.47)	165.14 (6.67)	0.164
Weight (kg), median (IQR)	64 (62–78)	74 (63–78)	56 (55–80)	63 (62–82)	0.507
BMI, median (IQR)	25.6 (23.3–28.9)	27 (24.9–28.9)	25.6 (23.3–30.5)	22.9 (21.8–27.4)	0.265
DM, n (%)	17 (80.95)	5 (71.43)	7 (100.00)	5 (71.43)	0.484
HT, n (%)	20 (95.24)	6 (85.71)	7 (100.00)	7 (100.00)	>0.999
Dyslipidemia, n (%)	20 (95.24)	6 (85.71)	7 (100.00)	7 (100.00)	>0.999
Smoking, n (%)	8 (38.09)	2 (28.57)	2 (28.57)	4 (57.14)	0.618
Alcohol, n (%)	4 (19.05)	1 (14.29)	-	3 (42.86)	0.263
EuroSCORE, mean (SD)	1.03 (0.3)	0.84 (0.17)	1.15 (0.23)	1.1 (0.4)	0.104
LVEF by echo, mean (SD)	51.14 (15.6)	55.7 (10.77)	58 (14.56)	39.71 (15.86)	0.051
Hemoglobin (g/dL), mean (SD)	12.75 (2.07)	13.21 (1.36)	11.17 (1.79)	13.86 (2.17)	0.031
HbA1c (%), median (IQR)	7.3 (6.2–7.9)	7.3 (6–7.9)	7.9 (6.9–7.9)	6.6 (6.1–7.8)	0.707
Creatinine (mg/dL), median (IQR)	1.06 (0.74–1.46)	1.06 (0.74–1.35)	1.46 (0.7–3.17)	1.04 (0.63–1.38)	0.299
Albumin (g/dL), mean (SD)	3.94 (0.54)	4.07 (0.42)	3.7 (0.61)	4.06 (0.57)	0.366
LDL cholesterol (mg/dL), mean (SD)	91.86 (30.36)	102.29 (30.78)	74.43 (27.09)	98.86 (29.22)	0.177

BMI: Body mass index, LVEF: Left ventricular ejection fraction, SD: Standard deviation, IQR: Interquartile range, LDL: Low-density lipoprotein, HbA1c: Glycated hemoglobin, DM: Diabetes mellitus, HT: Hypertension

Table 2: Intra- and postoperative data

Outcome	Total (n=21)	St. Thomas (n=7)	Del Nido (n=7)	Custodiol (n=7)	P
Clinical					
Number of grafts, median (IQR)	3 (3–4)	3 (3–3)	3 (3–3)	4 (4–4)	0.008
AOX time (min), median (IQR)	70 (61–76)	73 (68–86)	56 (43–61)	76 (65–112)	0.005
CPB time (min), median (IQR)	122 (98–133)	126 (122–140)	104 (91–106)	125 (91–151)	0.081
Volume of cardioplegia (mL), median (IQR)	1100 (700–1850)	600 (560–700)	1000 (1000–1500)	2000 (1850–2000)	<0.001
Intraoperative defib, n (%)	2 (9.52)	-	2 (28.57)	-	0.300
IABP, n (%)	1 (4.76)	-	1 (14.29)	-	>0.999
Complications, n (%)					
Fever	5 (23.81)	3 (42.86)	1 (14.29)	1 (14.29)	0.545
Low CO	2 (9.52)	1 (14.29)	1 (14.29)	-	>0.999
Perioperative MI	-	-	-	-	
Postoperative AF	7 (33.33)	3 (42.86)	3 (42.86)	2 (28.57)	0.817
Delirium	1 (5)	-	-	1 (14.29)	>0.999
Stroke	1 (4.76)	-	1 (14.29)	-	>0.999
AKI	7 (33.33)	-	4 (57.14)	3 (42.86)	0.115
Pleural effusion	1 (4.76)	-	1 (14.29)	-	>0.999
Pacemaker	1 (4.76)	1 (14.29)	-	-	>0.999
ICU stays (days), median (IQR)	4 (3–5)	5 (3–8)	4 (4–5)	4 (3–5)	0.055
Hospital stays (days), median (IQR)	13 (10–20)	15 (11–28)	18 (9–26)	10 (9–13)	0.095
Lab chemistry					
Creatinine (mg/dL), median (IQR)					
D0	1.19 (0.74–1.31)	1.19 (0.84–1.31)	1.5 (0.7–2.62)	1.13 (0.74–1.28)	0.407
D1	1.31 (0.86–1.68)	1.25 (0.86–1.35)	1.68 (0.82–3.71)	1.06 (0.62–2.16)	0.293
D2	1.19 (0.94–1.71)	1.19 (1–1.41)	1.65 (0.79–5.35)	1.05 (0.65–2.74)	0.370
D3	1.07 (0.83–1.47)	0.96 (0.83–1.27)	1.44 (0.77–5.28)	1.04 (0.54–1.47)	0.358
CK-MB (ng/mL), median (IQR)					
D0	58 (51–69)	61 (56–73)	54 (46–69)	56 (50–76)	0.839
D1	41 (30–53)	36 (28–46)	30 (27–53)	51 (41–60)	0.106
D2	25 (21–29)	22 (17–27)	27 (18–29)	29 (23–31)	0.223
D3	20 (15–24)	18 (12–22)	20 (13–26)	23 (15–28)	0.629
Troponin T (ng/mL), median (IQR)					
D0	421 (329–596)	423 (364–781)	329 (279–462)	421 (385–596)	0.563
D1	573 (331–1061)	366 (308–2026)	573 (306–868)	962 (436–1147)	0.834
D2	343 (265–639)	326 (187–976)	343 (265–639)	353 (267–693)	0.989
D3	273 (197–417)	210 (197–778)	274 (190–568)	273 (195–359)	0.953

AOX: Aortic cross clamp, CPB: Cardiopulmonary bypass, IABP: Intra-aortic balloon pump, CO: Cardiac output, AKI: Acute kidney injury, ICU: Intensive care unit, D: Postoperative day, MI: Myocardial infarction, IQR: Interquartile range, AF: Atrial fibrillation, CK-MB: Creatine kinase-MB or creatine kinase-myocardial band

Custodiol group

OPLS-DA showed significant changes in metabolic profiles on postoperative days 0 and 1 compared to the preoperative period [Figure 3c and d], as confirmed by permutation tests with *P* values of 0.013 and 0.022, respectively. No significant changes were observed on the following days. VIP score analysis [Supplementary Figure 1a and b] presented the significant differentially expressed metabolites (DEMs). After identifying these significant DEMs, we conducted univariate analysis, which showed notable changes in the concentrations of these metabolites, illustrated in box plots [Figures 4f and g].

On postoperative day 0, levels of isoleucine, valine, leucine, citrate, tyrosine, and phenylalanine decreased, while histidine and acetone levels increased significantly [Figure 4f]. On postoperative day 1, succinate levels increased, and citrate

levels decreased [Figure 4g]. Metabolic enrichment and pathway analysis revealed that key pathways, such as histidine, tyrosine, phenylalanine, and tryptophan metabolism, were affected on postoperative day 0 [Figure 4k]. The citrate cycle (TCA Cycle) was notably impacted on postoperative day 1 [Figure 4l].

Modified del Nido group

In the modified del Nido group, a significant metabolomic shift was observed only on postoperative day 0 [Figure 3e] with a *P* value of 0.049. VIP score analysis [Supplementary Figure 1c] and univariate analysis with box plot [Figure 4h] identified significant DEMs, with postoperative day 0 samples showing increased levels of acetone and decreased levels of phenylalanine and formate compared to preoperative samples. Pathway analysis revealed significant alterations

in phenylalanine, tyrosine, and tryptophan biosynthesis metabolism [Figure 4m]

St. Thomas group

In our study, St. Thomas cardioplegia was the only group that did not exhibit significant metabolomic changes after surgery (postoperative days 0–3) when compared to the preoperative baseline [Supplementary Figure 2].

Custodiol versus modified del Nido, Custodiol versus St. Thomas and modified del Nido versus St. Thomas

Analysis using OPLS-DA identified distinct metabolomic profiles between Custodiol and modified del Nido cardioplegia on operative day 0 [Figure 3f], with statistical significance ($P = 0.018$). Box plots [Figure 4i] indicated elevated levels of histidine and alanine in the Custodiol group, with histidine metabolism being the only significantly altered pathway [Figure 4n]. Comparisons between Custodiol and St. Thomas also revealed significant metabolomic differences on day 0 [Figure 3g] with a P value of 0.014, where histidine was the only elevated metabolite in the Custodiol group [Figure 4j]. No significant differences in postoperative metabolomic profiles were found between modified del Nido and St. Thomas [Supplementary Figure 3].

DISCUSSION

Our study confirms that different cardioplegia types result in distinct metabolomic responses in CABG patients, especially in the immediate postoperative period. Both Custodiol and modified del Nido cardioplegia induce significant postoperative metabolomic shifts, whereas St. Thomas cardioplegia does not cause significant alterations.

Clinical outcomes and cardiac enzyme level

Many studies, including ours, report no significant differences in postoperative morbidity and mortality among CABG patients using various cardioplegia types.^[7,8] Custodiol, consistent with earlier research,^[9] is associated with shorter hospital stays. Both modified del Nido and Custodiol may reduce operative time due to their long-acting effects.^[10] In our study, Custodiol required the highest administration volume, aligning with other research.^[11] We found no significant differences in postoperative cardiac enzyme levels among the groups, in line with earlier studies.^[12] All types of cardioplegia exhibited the expected postoperative pattern in nature, with CK-MB peaking on day 0 and Troponin T reaching its highest level on postoperative day 1, reflecting myocardial injury following surgery. However, some research suggests modified del Nido might lower peak Troponin T and CK-MB levels compared to other solutions.^[13]

Postoperative metabolomic shifts and implications in the Custodiol group

Our investigation revealed significant metabolomic alterations in patients receiving Custodiol, particularly on postoperative days 0 and 1. We observed reductions in isoleucine, valine, and leucine, essential branched-chain amino acids (BCAAs)

involved in protein synthesis and energy production, suggesting increased utilization due to surgical stress [Figure 5]. Although direct research on BCAAs and cardiac surgery is limited, dysregulation of these amino acids is associated with cardiovascular pathology and recognized as modifiers of coronary heart disease risk factors.^[14]

In addition, decreases in tyrosine and phenylalanine, essential amino acids crucial for tissue repair processes postsurgery,^[15] were observed. Perturbation of these pathways may contribute to an exaggerated inflammatory response postsurgery, cognitive dysfunction, and delayed healing.^[16,17] Furthermore, tyrosine is a precursor for nitric oxide synthesis, critical for tissue perfusion and healing postsurgery.

Increased levels of histidine and acetone were observed. Histidine, a component of Custodiol solution used for buffering and free radical scavenging, typically rises postoperatively.^[18-20] The observed elevation in histidine levels is likely due to its presence in the Custodiol solution, which may not be fully utilized to meet increased demand. Under normal physiological conditions, histidine is required for tissue repair, and its concentrations may also reduce in response to inflammation, processes commonly associated with surgical intervention.^[21,22] Acetone, a metabolite of lipid metabolism, increases after surgery, likely due to heightened lipid breakdown and altered metabolic states. Ketone bodies, including acetone, serve as an important alternative energy source during periods of reduced glucose availability, such as in prolonged surgery. Elevated levels of acetone and other ketone bodies may indicate a shift in energy metabolism to support the heart and other tissues during the stress of surgery. In addition, a previous study found that exhaled acetone concentrations directly correlate with oxidative stress markers, such as C-reactive protein and troponin-T.^[23]

The significant alterations on postoperative day 1 involved the levels of citrate and succinate. These citric acid cycle intermediates play crucial roles in cellular energy metabolism. Decreases in citrate and increases in succinate levels may indicate disruptions in citric acid cycle activity or mitochondrial function.^[24] Succinate accumulation during ischemia contributes to ischemia-reperfusion injury and metabolic stress, leading to the generation of reactive oxygen species (ROS) upon reperfusion. Studies have shown that inhibiting ischemic succinate accumulation can improve ischemia-reperfusion injury outcomes in murine models of heart attack.^[24]

Postoperative metabolomic shifts and implications in the modified del Nido group

Patients receiving modified del Nido cardioplegia exhibited significant metabolomic changes on postoperative day 0, including increased acetone levels and decreased phenylalanine levels. The reduction in phenylalanine may indicate altered neurotransmitter synthesis or protein metabolism due to the surgery.^[25]

In addition, decreasing in formate levels indicates potential alterations in cellular metabolic pathways, such as nucleotide synthesis and methylation reactions,^[26] with implications for cellular proliferation and epigenetic regulation.^[27] These metabolic changes may contribute to postoperative complications such as impaired wound healing, oxidative stress, or neurological dysfunction.^[28]

Comparison of metabolomic profiles between the Custodiol, modified del Nido, and St. Thomas groups

Histidine and alanine levels were higher in the Custodiol group compared to the modified del Nido group, and histidine levels were similarly elevated in the Custodiol group relative to the St. Thomas group. Metabolomic pathway analysis revealed that histidine metabolism was the only significantly altered pathway between the groups. This analysis provides a comprehensive view of the broader metabolic shifts associated with different cardioplegia solutions, highlighting underlying metabolic processes that may not be immediately apparent from individual metabolite analysis. As previously discussed, the increase in histidine levels in the Custodiol group can be attributed to histidine being a component of the Custodiol solution. These changes in histidine levels might also indicate alterations in histamine metabolism in response to tissue injury or inflammation. Given histidine is important in mitigating oxidative stress in humans.^[29] However, it is important to note that the interpretation of histidine levels in this context is limited. The observed increase may not necessarily reflect a true metabolic response, as it could be influenced by the exogenous histidine in the cardioplegia solution.

Alanine was elevated in the Custodiol group, suggesting increased utilization for gluconeogenesis or energy production postsurgery. Hou *et al.* demonstrated that alanine could reduce cardiac infarction size, lipid peroxidation, inflammation, ROS levels, and apoptosis, underscoring its importance in cardiac health and stress reduction.^[30]

To our knowledge, this is the first study to directly compare postoperative metabolomic profiles in cardiac surgery patients to evaluate the effects of different cardioplegia types. However, several limitations must be acknowledged. As a pilot study, it is constrained by a small sample size and a predominance of low-risk patients, which may limit the ability to detect differences and their clinical significance. In addition, the inherent complexity of metabolomic responses, influenced by factors such as cardiopulmonary bypass effects and surgical stress, can complicate result interpretation. Despite these limitations, our findings provide valuable insights into postoperative metabolomic disturbances and their physiological implications. These insights may contribute to optimizing cardioplegia strategies by refining or developing formulations to mitigate metabolic alterations associated with surgical procedures. Furthermore, targeted pre- and postoperative care, including enhanced nutritional support and supplementation to prevent specific metabolite depletion

postoperatively, may improve clinical outcomes in cardiac surgery.

CONCLUSION

Different cardioplegia solutions induced distinct metabolomic changes postoperatively. St. Thomas showed no significant alterations, while Custodiol and modified del Nido induced substantial shifts, with Custodiol causing the most pronounced changes within the first 2 days. Key metabolites affected included histidine, succinate, acetone, and amino acids. In contrast, modified del Nido cardioplegia primarily affected the metabolism of acetone, phenylalanine, and formate on postoperative day 0. These differentially expressed metabolites were mainly associated to protein and energy metabolism, oxidative stress, and inflammation. Significant differences in metabolomic profiles between the groups were noted on day 0, with key metabolites such as histidine and alanine. The findings confirm that distinct cardioplegia solutions lead to unique metabolic states.

Availability of data and materials

The data underlying this article will be shared on reasonable request to the corresponding author.

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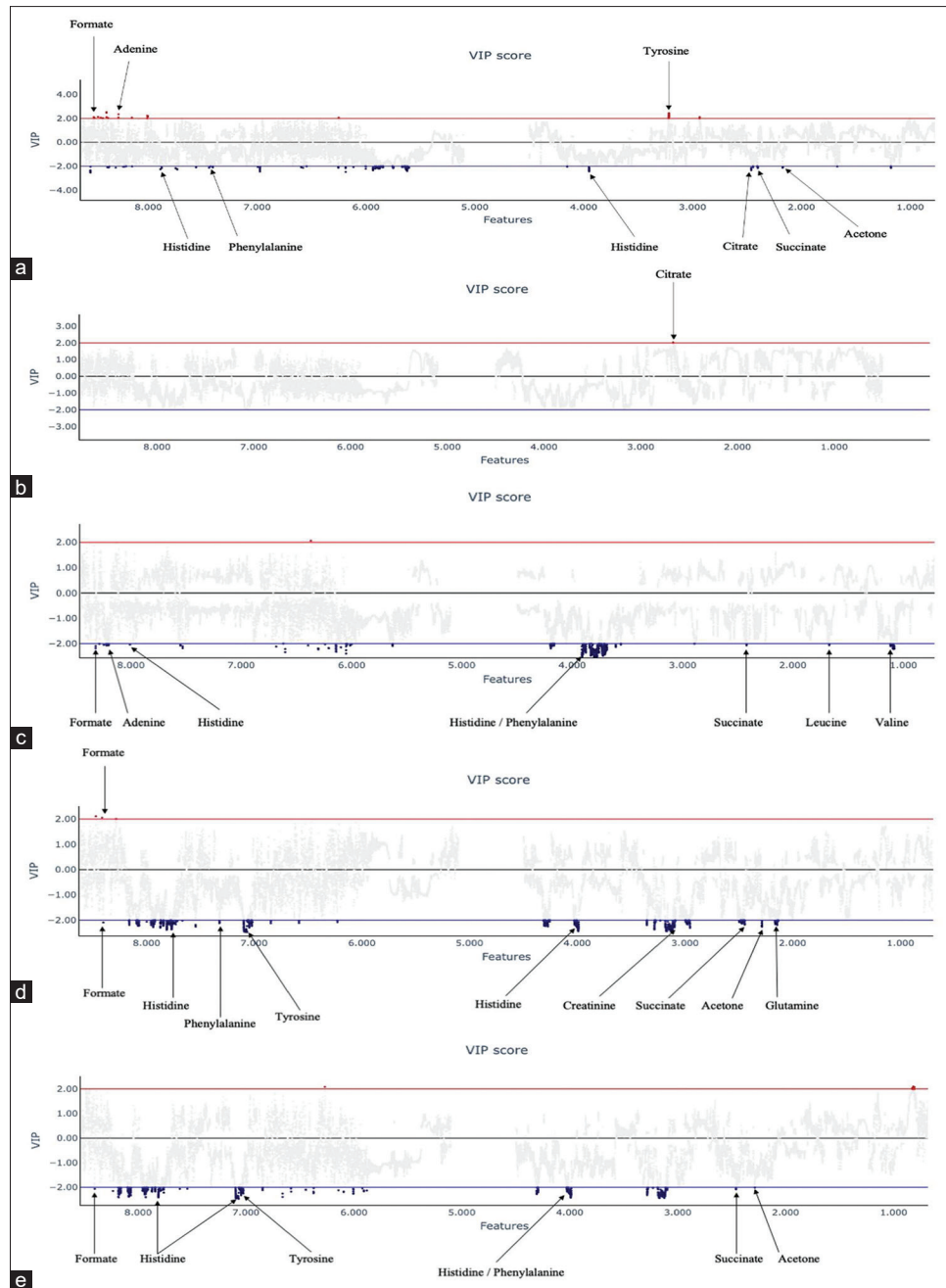
Conflicts of interest

There are no conflicts of interest.

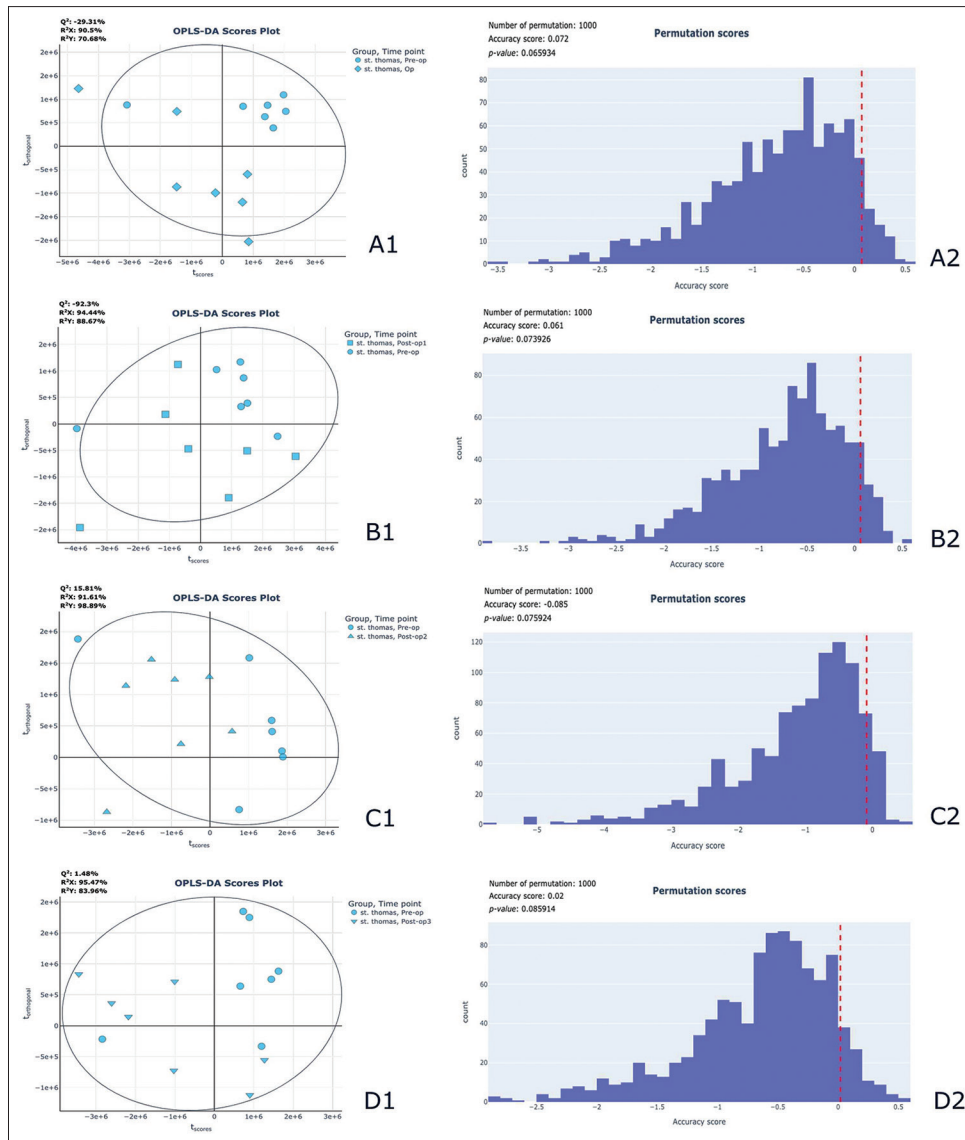
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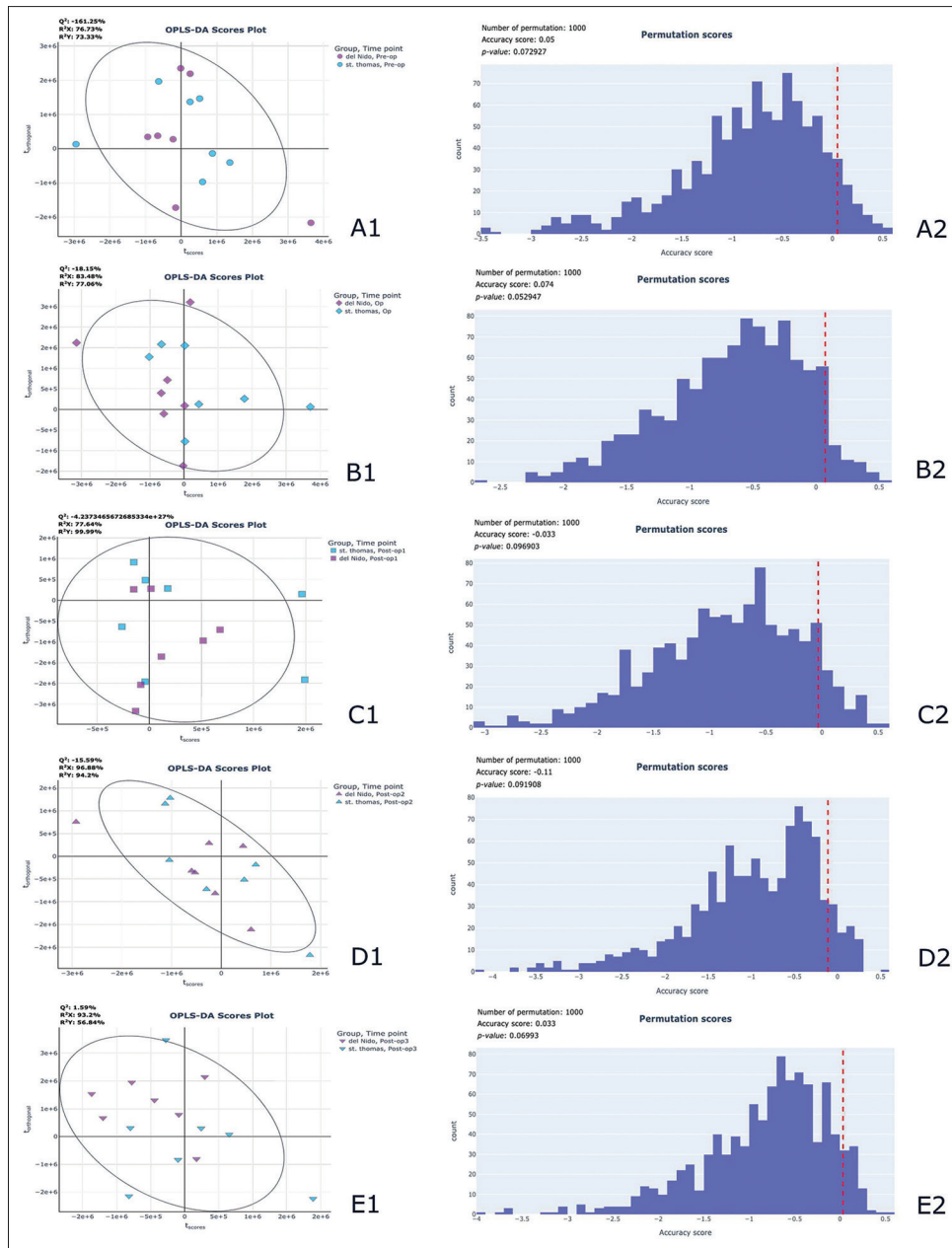
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Supplementary Figure 1: The Variable Importance in Projection (VIP) score plot. The comparisons include (a) Custodiol preoperative versus postoperative day 0, (b) Custodiol preoperative versus postoperative day 1 (c) Del Nido preoperative versus postoperative day 0, (d) Custodiol versus del Nido on postoperative day 0, (e) Custodiol versus St. Thomas on postoperative day 0. It's important to note that metabolites with VIP scores above certain threshold, set at 2, are considered significant and selected for further analysis. VIP: Variable importance in projection.



Supplementary Figure 2: The orthogonal partial least squares discriminant analysis and permutation test results for the St. Thomas receiving group, comparing the preoperative period with postoperative day 0 (A1 and A2), day 1 (B1 and B2), day 2 (C1 and C2), and day 3 (D1 and D2).



Supplementary Figure 3: The orthogonal partial least squares discriminant analysis and permutation test results comparing the modified del Nido and St. Thomas receiving group at the preoperative period (A1 and A2) with postoperative day 0 (B1 and B2), day 1 (C1 and C2), day 2 (D1 and D2), and day 3 (E1 and E2).

Supplementary Table 1: Postoperative ventilator support and inotropic drug use

Outcome	Total (n=21)	St. Thomas (n=7)	Del Nido (n=7)	Custodiol (n=7)	P
Ventilator (days), n (%)					
D0	13 (61.9)	2 (28.57)	6 (85.71)	5 (71.43)	0.140
D1	7 (33.33)	2 (28.57)	3 (42.86)	2 (28.57)	>0.999
D2	5 (23.81)	2 (28.57)	2 (28.57)	1 (14.29)	>0.999
D3	5 (23.81)	3 (42.86)	2 (28.57)	-	0.292
Dobutamine (µg/kg/min), median (IQR)					
D0	0 (0–3)	0 (0–0)	0 (0–3)	0 (0–2)	0.131
D1	0 (0–5)	0 (0–0)	0 (0–5)	0 (0–0)	0.123
D2	0 (0–2)	0 (0–2)	0 (0–0)	0 (0–0)	0.368
D3	0 (0–2)	0 (0–0)	0 (0–2)	0 (0–0)	0.368
Adrenaline (µg/kg/min), median (IQR)					
D0	0 (0–0.21)	0.01 (0–0.05)	0 (0–0.21)	0 (0–0.02)	0.203
D1	0 (0–0.20)	0 (0–0)	0 (0–0.20)	0 (0–0.07)	0.589
D2	0 (0–0.01)	0 (0–0.01)	0 (0–0)	0 (0–0)	0.368
D3	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	
Levophed (µg/kg/min), median (IQR)					
D0	0 (0–0.03)	0 (0–0)	0 (0–0.09)	0 (0–0.04)	0.402
D1	0 (0–0.05)	0 (0–0.05)	0 (0–0.09)	0 (0–0.05)	0.973
D2	0 (0–0.03)	0.03 (0–0.05)	0 (0–0.04)	0 (0–0)	0.085
D3	0 (0–0)	0 (0–0.04)	0 (0–0.02)	0 (0–0)	0.167

IQR: Interquartile range