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# Identification of metabolomic profile related to adult Fontan pathophysiology

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ABSTRACT

*Background:* Metabolic disorders are important pathophysiologies that can cause multiple organ dysfunction and worsen prognosis in Fontan patients. This study aimed to comprehensively evaluate the metabolomic profile of adult Fontan patients and characterize its pathophysiology in relation to 2 control groups.

*Methods and Results:* We performed metabolomic analysis of 31 plasma samples using capillary electrophoresis time-of-flight mass spectrometry. This observational cross-sectional study compared plasma metabolites of 14 heterogeneous adult Fontan patients with those of control groups, including 9 patients with congenital heart disease after biventricular repair and 8 normal healthy controls. Fontan patients exhibited significant differences in intermediate metabolite concentrations related to glycolysis, the tricarboxylic acid (TCA) cycle, and the urea cycle. The plasma concentrations of lactic acid, 2-oxoglutarate, isocitric acid, malic acid, *cis*-aconitic acid, arginine, citrulline, and the ratio of ornithine/citrulline showed significantly differences among the groups. Multiple logistic regression analysis with a stepwise selection-elimination method identified 2-oxoglutaric acid (odds ratio [OR] 1.98, 95% confidence interval [CI] 1.05–3.76) and *cis*-aconitic acid (OR 2.69, 95% CI 1.04–6.99) as independently associated with Fontan patients. After adjustment for the covariates of age and gender, 2-oxoglutaric acid (OR 1.97, 95% CI 0.98–3.93) and *cis*-aconitic acid (OR 3.88, 95% CI 0.99–15.2) showed remarkable relationships with Fontan patients.

*Conclusions:* The present findings suggest that abnormalities in the TCA cycle and amino acid metabolism are distinguishing features in the pathophysiology of Fontan patients. Future metabolomic studies will assist in developing biomarkers for the early prediction of "silent" Fontan pathophysiologies.

## 1. Introduction

The introduction of various modifications to the Fontan operation and advances in perioperative management have markedly improved the postoperative survival of patients with single ventricular physiology [1]. The majority of these patients now reach adulthood; however, morbidity and mortality remain high [2–4]. After the Fontan operation, the palliative nature of the procedure is associated with various non-cardiac comorbidities, including renal and liver dysfunctions, which can have an adverse effect on survival [5–7]. Glucose and lipid metabolism disorders have also been described in patients with Fontan circulation [8,9]. Metabolic disorders are important pathophysiologies that are related to asymptomatic multiple organ dysfunction and may diminish prognosis [10].

Metabolites are the end products of all processes occurring in cells. Metabolite profiling in various diseases has helped characterize the adaptation of biological systems to pathological conditions and contributed to the improvement of diagnostic and therapeutic methods [11–13]. Therefore, metabolomics represents a potential tool for identifying biomarkers to understand the pathophysiological mechanism of cardiovascular diseases [14]. Several studies have addressed the metabolic alterations in patients with such cardiovascular conditions as coronary artery disease, arrhythmia, and heart failure [15,16]. Recently, metabolomic analysis has also been used in patients with congenital heart disease (CHD) to investigate predictors [17]. Cedars et al. revealed several metabolomic variations associated with cardiac function and

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health status in anatomically heterogeneous adults with CHD [18]. There are few metabolomic studies on adult patients with Fontan circulation [19,20], and the association between their metabolomic profile and unique pathophysiology remains uncertain. Metabolomic profile characterization may unlock the identification of diagnostic markers and/or therapeutic targets of asymptomatic multiple-organ disorders in Fontan patients.

This study aimed to comprehensively evaluate the metabolomic profile of adult Fontan patients and characterize its pathophysiology in relation to 2 control groups.

## 2. Materials and methods

### 2.1. Participants

Between April 2018 and February 2019, we recruited adult patients (≥18 years old) with single ventricular physiology after the Fontan procedure (ex., hypoplastic left heart syndrome, tricuspid atresia, pulmonary atresia, etc.). As control groups, patients after biventricular repair (biventricular group), such as for tetralogy of Fallot or ventricular septal defect, and healthy subjects with no significant lung or heart problems at a routine physical checkup (normal group) were enrolled. Patients receiving the Fontan procedure or biventricular repair were recruited from the Pediatrics department or Adult Congenital Heart Disease Center at Shinshu University Hospital or from Nagano Children's Hospital. All study participants were not pregnant or lactating, had no malignancy, and were free from intravenous medications. Participants with such complications as arrhythmia, atrioventricular valve regurgitation, and protein-losing enteropathy (PLE) were not excluded from this study since they were well managed by oral medications and exhibited no acute exacerbation, and thus were considered clinically stable. The demographic data, diagnosis, history of operations, medications, cardiac function, and complications of the 2 patient groups were collected from medical records.

In Fontan patients, cardiac catheterization findings including cardiac index (CI; L/min/$m^2$), systemic ventricular ejection fraction (EF), and central venous pressure (CVP) were recorded. CI was estimated by the Fick principle assuming that right and left pulmonary arterial saturations were equal in Fontan patients since it was clinically difficult to accurately measure flow distribution in the bilateral pulmonary arteries. EF was calculated angiographically or echocardiographically. In addition to brain natriuretic peptide levels, various blood biomarkers were measured, including serum albumin, total bilirubin, alanine aminotransferase, γ-glutamyltransferase, uric acid, creatinine, and glucose. Type IV collagen, hyaluronic acid, and Mac-2 binding protein were measured in 10 Fontan patients as liver fibrosis markers. Recently administered medications included diuretics, anti-coagulant agents, angiotensin-converting enzyme inhibitor (ACE-I), angiotensin II receptor blocker (ARB), β-blockers, anti-hyperuricemia drugs, pulmonary artery dilators, and anti-arrhythmic agents. The β-blockers included carvedilol, metoprolol, and propranolol. Clinical information on liver ultrasonography, magnetic resonance imaging (MRI), and biopsy findings were obtained from medical records for FALD assessment.

The protocol of this study was approved by the Institutional Review Board of Shinshu University School of Medicine (authorization number: 4055) and was conducted in accordance with the Declaration of Helsinki. All participants provided written informed consent for participation in this study.

### 2.2. Blood sample processing

Peripheral blood samples were drawn from a peripheral vein with the participant in a fasting state (>3h without any meal or nutritious drink). Blood plasma was isolated by centrifugation at 3500 *g* for 10 min, and then stored at −80 °C until use. Metabolite extraction and analysis were conducted at Human Metabolome Technologies, Inc. (Tsuruoka, Yamagata, Japan) as described in detail in the Supplementary Appendix. Metabolome analysis was performed using capillary electrophoresis time-of-flight mass spectrometry (CE-TOFMS) based on methods described previously [21–24].

### 2.3. Statistical analysis

For each metabolite, the relative area was defined as the relative concentration of that metabolite. As CE-TOFMS was able to identify even small amounts of relative areas of metabolites, several metabolites were not uniformly detected across the samples. Since these data could not be distinguished as being due to a subthreshold value or a zero value in this system, one half of the minimum measure for that sample was imputed [24]. CE-TOFMS analysis using the current system enabled measurement of the absolute quantities of the pre-determined major metabolites in each sample on the basis of the peak area of internal controls. The quantity of those metabolites could therefore reliably be compared across different experimental batches. The Kruskal–Wallis test followed by post hoc (Dunn–Bonferroni) analysis were used to evaluate the absolute concentrations of the major metabolites among the Fontan, biventricular, and normal groups. Demographic data were also compared among the groups using the Kruskal–Wallis test, with categorical data being compared with Fisher's exact test. We also performed multiple logistic regression with a stepwise selection-elimination method to select metabolites with a strong potential for distinguishing Fontan patients from the other controls. The threshold for statistical significance was set to $P < 0.05$ for all analyses. Statistical testing was conducted using SPSS software version 27.0 (IBM, Armonk, NY, USA).

## 3. Results

The demographic characteristics of the subject groups are summarized in Table 1 and Supplementary Table S1. Fourteen patients with single-ventricular physiology after the Fontan procedure, 9 patients with biventricular repair, and 8 normal controls were recruited. The 3 groups did not differ significantly in terms of age, gender, and BMI (Supplementary Table S1).

The majority of Fontan patients had double outlet right ventricle, followed next by tricuspid atresia and mitral stenosis or mitral atresia (Table 1). Ventricular morphology was 7 in the right ventricle and 6 in the left ventricle, with 1 unknown. Most Fontan patients had undergone 1 or more palliative surgeries, including the Blalock–Taussig shunt and the Glenn procedure. Eleven patients had received total cavopulmonary connection (TCPC). We observed moderate to severe atrioventricular valve regurgitation in 2 patients as well as tachyarrhythmia and bradyarrhythmia in 3 patients each, whose hemodynamics were stable due to oral medication or pacemaker insertion. One patient had a history of PLE but was maintained in remission by oral steroid treatment. No patient had plastic bronchitis. Regarding metabolism-related pathologies, there were no cases of diabetes, although 1 patient had hyperuricemia and hyperlipidemia, the former of which was under treatment. The hemodynamic results for CVP, CI, and EF are presented in Table 1. Biochemical markers and liver fibrosis markers were within normal limits in all patients. The administration of medications, including diuretics, anti-coagulant agents, ACE-I/ARB, β-blockers, and anti-arrhythmic agents is described in Table 1. Steroid therapy was given to 2 patients: 1 for PLE and 1 for destructive thyroiditis caused by amiodarone.

Eleven patients underwent liver ultrasound and 8 patients received elastography (Table 2). No patients underwent MRI or biopsy of the liver. The most common ultrasound findings were heterogeneous parenchymal echotexture (N = 8), surface irregularity/nodularity (N = 8), and hepatic vein dilatation (N = 5). Hyperechogenic lesions were present in 3 patients, and sonographic signs of liver congestion and cirrhosis were detected in 7 and 5 patients, respectively. Median liver stiffness value was 18.3 kPa (range: 12.0–28.5 kPa), which was well

**Table 1**
Demographic and clinical characteristic of Fontan patients.

| | Fontan group (N = 14) |
|---|---|
| Age (years), mean ± SD | 29.5 ± 8.9 |
| Gender (male), N (%) | 9 (64) |
| BMI (kg/m$^2$), mean ± SD | 22.1 ± 5.8 |
| Diagnosis, N (%) | |
| TA | 3 (22) |
| DORV | 7 (50) |
| MS/MA | 2 (14) |
| ccTGA | 1 (7) |
| PAIVS | 1 (7) |
| Systemic ventricle, N (%) | |
| RV | 7 (50) |
| LV | 6 (43) |
| Unknown | 1 (7) |
| Number of operations, median (range) | 2.5 (1–7) |
| APC/TCPC, N | 3/11 |
| Heterotaxy, N (%) | 2 (14) |
| PLE, N (%) | 1 (7) |
| Atrioventricular valve regurgitation, N (%) | |
| None to slight | 12 (86) |
| Moderate | 1 (7) |
| Severe | 1 (7) |
| Tachyarrhythmia/bradyarrhythmia, N | 3/3 |
| NYHA class, median (range) | 1 (1–2) |
| Hemodynamics, mean ± SD | |
| CVP (mmHg) | 10.2 ± 2.9 |
| CI (L/min/m$^2$) | 3.3 ± 1.5 |
| EF (%) | 54.0 ± 9.4 |
| Hemoglobin (g/dL) | 16.7 ± 2.3 |
| $SpO_2$ (%) | 92.3 ± 2.7 |
| Neurohumoral factors | |
| BNP (pg/mL), mean ± SD | 49.6 ± 59.3 |
| Biochemical variables, mean ± SD | |
| Albumin (g/dL) | 4.6 ± 0.4 |
| Total bilirubin (mg/mL) | 1.1 ± 0.5 |
| ALT (U/L) | 29.2 ± 22.6 |
| GGT (U/L) | 104.2 ± 66.1 |
| Uric acid (mg/mL) | 5.3 ± 1.8 |
| Creatinine (mg/dL) | 0.71 ± 0.11 |
| Glucose (mg/dL) | 93.6 ± 19.4 |
| Liver fibrosis markers, mean ± SD | |
| Type IV collagen (ng/mL) | 7.3 ± 1.2 |
| Hyaluronic acid (ng/mL) | 57.6 ± 33.5 |
| Mac-2 binding protein (COI) | 0.38 ± 0.32 |
| Medications, N (%) | |
| Diuretics | 7 (50) |
| Anticoagulants | 11 (79) |
| ACEI/ARB | 8 (57) |
| β-blockers | 2 (14) |
| Antihyperuricemia drugs | 2 (14) |
| Pulmonary artery dilators | 4 (28) |
| Anti-arrhythmic drugs | 2 (14) |
| Steroids | 2 (14) |

SD, standard deviation; BMI, body mass index; TA, tricuspid valve atresia; DORV, double outlet right ventricle; MS, mitral stenosis; MA, mitral atresia; ccTGA, congenital corrected transposition of great arteries; PAIVS, pulmonary atresia with intact ventricular septum; TOF, tetralogy of Fallot; VSD, ventricular septal defect; RV, right ventricle; LV, left ventricle; APC, atriopulmonary connection; TCPC, total cavopulmonary connection; PLE, protein-losing enteropathy; NYHA, New York Heart Association; CVP, central venous pressure; CI, cardiac index; EF, systemic ventricular ejection fraction; $SpO_2$, percutaneous oxygen saturation; BNP, brain natriuretic peptide; ALT, alanine aminotransferase; GGT, γ-glutamyltransferase; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker.

above the adult liver stiffness cut-off value of 7.71 kPa for significant liver fibrosis [25].

CE-TOFMS successfully identified 225 metabolites (124 and 101 metabolites for cationic and anion modes, respectively) and quantified 66 metabolites (39 and 27 cationic and anion modes, respectively). Thirteen metabolites showed significantly different concentrations between the Fontan group and 2 control groups (Supplementary Table S2).

To identify metabolites that could discriminate Fontan patients from the controls, we selected candidates associated with glycolysis, the tricarboxylic acid (TCA) cycle, the urea cycle, and glutamine metabolism (Fig. 1 and Table 3). Regarding glycolysis, there was a significant difference among the 3 groups for the concentration of lactic acid ($P = 0.041$). In the TCA cycle, the concentrations of organic acids, including 2-oxoglutarate, isocitric acid, malic acid, and *cis*-aconitic acid, showed significant differences among the groups (all $P < 0.05$). For the urea cycle, there were significant differences in the concentrations of arginine and citrulline among the groups (both $P < 0.05$). Consequently, the ratio of ornithine/citrulline, reflecting metabolic activity in the urea cycle, was significantly different among the groups ($P = 0.010$). Regarding glutamine metabolism, glutamate and glutamine did not exhibit significant differences among the groups.

**Table 2**
Liver ultrasound findings in Fontan patients.

| | Median (range) | No (%) abnormal |
|---|---|---|
| Liver ultrasound findings | | |
| Hepatomegaly | | 2/11 (18.2) |
| Splenomegaly | | 2/11 (18.2) |
| Heterogeneous parenchymal echotexture | | 8/11 (72.7) |
| Surface irregularity/nodularity | | 8/11 (72.7) |
| Hyperechogenic lesions | | 3/11 (27.3) |
| Hepatic vein dilatation | | 5/11 (45.5) |
| Transient elastography | | |
| Liver stiffness (kPa) | 18.3 (12.0–28.5) | 8/8 (100) |

Multiple logistic regression analysis with a stepwise selection-elimination method was performed to evaluate the associations between 8 metabolite parameters and Fontan patients. We observed that 2-oxoglutaric acid (odds ratio [OR] 1.98, 95% confidence interval [CI] 1.05–3.76) and *cis*-aconitic acid (OR 2.69, 95% CI 1.04–6.99) were independently associated with Fontan patients (Table 4, Model 1). After adjustment for the covariates of age and gender, 2-oxoglutaric acid (OR 1.97, 95% CI 0.98–3.93) and *cis*-aconitic acid (OR 3.88, 95% CI 0.99–15.2) showed remarkable relationships with Fontan patients (Table 4, Model 2).

## 4. Discussion

We herein present the metabolome analysis of adult Fontan patients with various ventricular morphologies and Fontan-specific complications. Our study revealed that the concentrations of organic acids related to the TCA cycle (2-oxoglutaric acid and *cis*-aconitic acid) were remarkably associated with the metabolomic characterization of Fontan patients from patients receiving biventricular repair for congenital heart disease and normal controls.

Fontan circulation is characterized by chronically high CVP, low cardiac output, and mild hypoxemia. *cis*-aconitic acid and 2-oxoglutarate are major intermediates in the TCA cycle that perform a central role in energy metabolism [26]. Serum 2-oxoglutaric acid has been associated with a poor prognosis in heart failure patients, which was characterized by alterations in energy metabolism [27,28]. Recent findings using metabolomics to investigate protein alterations during cardiac ischemia have also shown decreases in several constituents of the TCA cycle [29]. Those findings suggest that the metabolic condition of the myocardium and/or other tissue is at least in part reflected in plasma metabolites, which can indicate altered myocardial cell energy metabolism as seen in biventricular patients with heart diseases. Javrushyan et al. reported that chronic hypoxia increased the activities of key TCA cycle enzymes in the rat heart but decreased them in the liver and brain [30]. It is therefore possible that TCA cycle activation in the heart is necessary since cardiac output must be increased to compensate for the diminished arterial $O_2$ saturation caused by hypoxia.

Late complications in Fontan patients also arise in non-

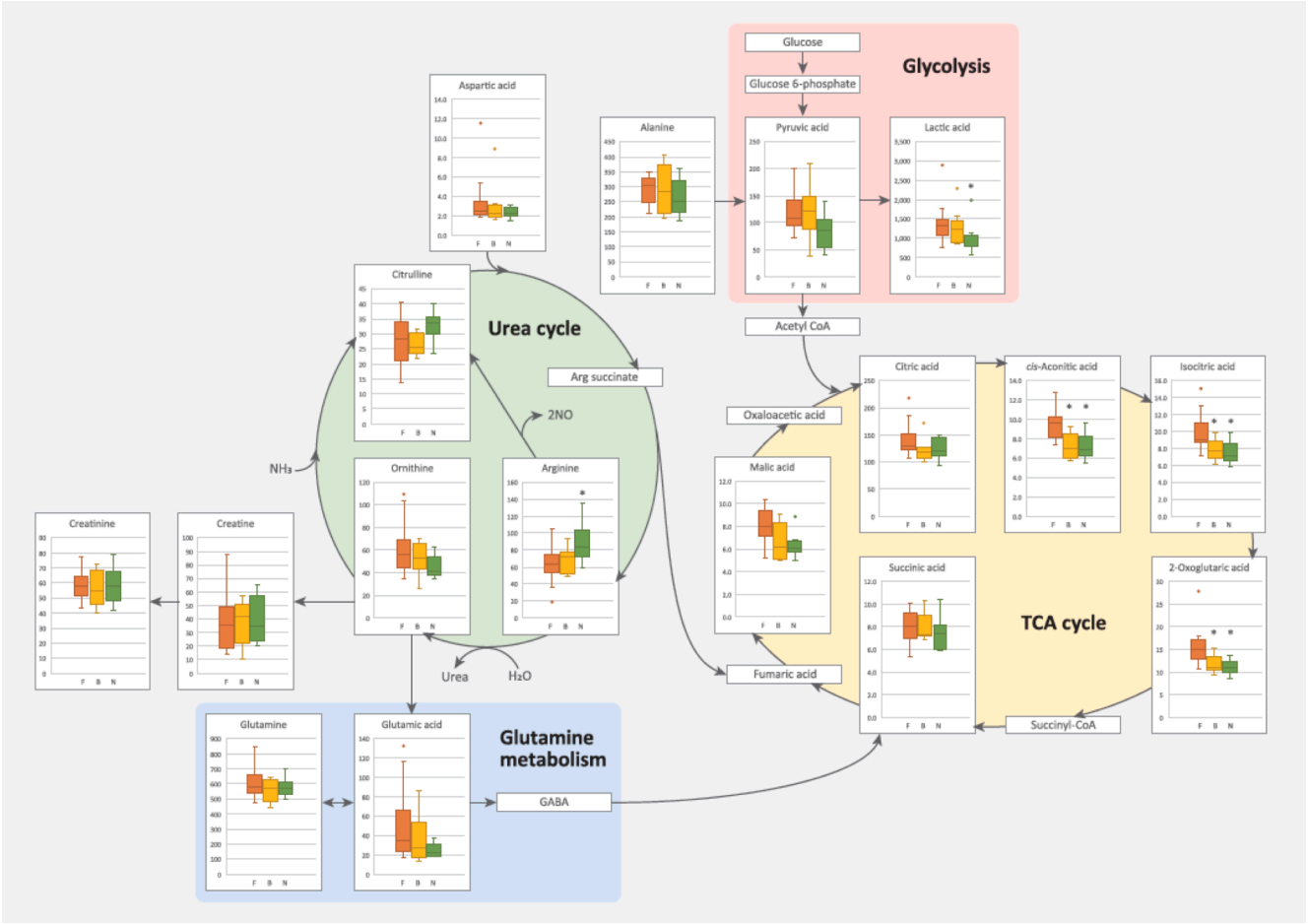


**Fig. 1.** Metabolic pathway map of the quantified metabolite concentrations, including those for glycolysis, the tricarboxylic acid cycle, the urea cycle, and glutamine metabolism, in the Fontan (F) group, the biventricular repair (B) group, and normal controls (N). Box-and-whisker plots of the plasma concentrations of metabolites involved in energy metabolism in the subject groups. The colored plots denote the F group (red), the B group (yellow), and the N group (green). Horizontal lines indicate the minimum, maximum, median, and first and third quartiles. All concentration units are μM. $^{*}P < 0.05$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

cardiovascular organ systems. In the Fontan anatomy, elevated CVP and non-pulsatile flow lead to a state of passive venous congestion secondary to increased hepatic afterload. Decreased cardiac output and increased CVP ultimately result in chronic hepatic congestion and cirrhosis [7]. Fontan-associated liver disease (FALD) is an extra-cardiac complication that may induce substantial comorbidities and premature mortality [5,7]. In FALD, standard serologic liver tests correlated poorly with the degree of liver disease, unlike in non-Fontan liver disease [5]. Furthermore, liver function testing is not useful until end-stage disease is present, and ultrasound/advanced imaging are poorly validated in FALD [7]. Thus, a better prognostic marker to detect early-stage FALD is necessary. A previous study reported high levels of plasma TCA cycle metabolites, including *cis*-aconitic acid and citric acid, in patients with hepatic insulin-sensitive non-alcoholic fatty liver disease [31]. Additionally, amino acid imbalances were observed in the plasma of patients with advanced cirrhosis, which interfered with TCA cycle mediators [32]. The present study revealed that the concentrations of organic acids related to the TCA cycle were significantly higher in Fontan patients as compared with those in controls. Although their biochemical and liver fibrosis markers were almost normal, most of the patients who underwent ultrasonography had abnormal findings, and the liver stiffness values determined in all 8 patients were high. This result may help detect early-stage metabolic disorders in the liver and assist in the detection of asymptomatic liver dysfunction, which are difficult to identify with conventional serum biochemical and liver fibrosis markers alone. The results of longitudinal metabolomic studies in collaboration with other non-invasive testing, such as liver transient elastography and MRI, will presumably add to our diagnostic armamentarium in Fontan patients.

Lastly, previous studies on adult Fontan patients with a dominant left ventricle reported that the observed perturbations in amino acid, phospholipid, and acylcarnitine metabolomes suggested altered cell energy metabolism, oxidative stress, endothelial dysfunction, and aberrant cell signaling as found in biventricular patients with congestive heart failure [15,16]. In contrast, the present study targeted adult Fontan patients exhibiting a wide variety of structural abnormalities, medications, and complications, and thus was considered to represent the heterogeneous Fontan population.

## 5. Limitations

This study had several limitations. The subject group sizes were small, which limited the extent to which our findings could be generalized. Second, dietary intake affecting metabolism was not unified, nor was the diurnal variation of metabolites taken into consideration. Third, many complicated heart defect patients take multiple drugs postoperatively, and the metabolic effects of such combinations have not been identified. Time-course analyses of metabolomic profiles in Fontan patients might help improve diagnostic accuracy.

**Table 3**
Comparisons of quantified metabolite concentrations, including those for glycolysis, the tricarboxylic acid cycle, the urea cycle, and glutamine metabolism, between the Fontan and control groups.

| | Concentration (μM) | | | | | | *P*-value * | *P*-value for Fontan vs. control 1 † | *P*-value for Fontan vs. control 2† | *P*-value for control 1 vs. control 2 † |
|---|---|---|---|---|---|---|---|---|---|---|
| Metabolite | Fontan group (N = 14) | | Control 1 (N = 9) Biventricular group | | Control 2 (N = 8) Normal control group | | | | | |
| | Mean (SD) | Median (range) | Mean (SD) | Median (range) | Mean (SD) | Median (range) | | | | |
| Alanine | 292.3 (43.5) | 307 (208–346) | 285.4 (81.5) | 284.4 (208.2–373.7) | 263.5 (59.0) | 250 (189–360) | 0.47 | – | – | – |
| Lactic acid | 1405.4 (517.0) | 1407.7 (760.8–2887.9) | 1260.8 (453.2) | 1228.5 (839.0–2279.6) | 966.5 (439.8) | 808.2 (770.9–1063.6) | **0.041** | 1.00 | **0.036** | 0.27 |
| Pyruvic acid | 121.7 (39.7) | 108.0 (72.6–201.2) | 119.9 (49.2) | 109.1 (37.7–210.0) | 83.6 (40.8) | 83.8 (40.8–130.3) | 0.113 | – | – | – |
| 2-Oxoglutaric acid | 15.6 (4.1) | 14.9 (10.6–27.9) | 11.7 (2.4) | 11.0 (9.2–15.2) | 11.0 (20.1) | 10.8 (8.5–13.7) | **0.002** | **0.031** | **0.003** | 1.00 |
| Citric acid | 139.3 (30.0) | 128.6 (106.8–218.0) | 120.5 (21.3) | 117.7 (100.8–171.4) | 124.1 (1.6) | 120.0 (92.5–149.3) | 0.125 | – | – | – |
| Isocitric acid | 9.9 (1.5) | 9.0 (7.1–15.1) | 7.9 (1.2) | 7.8 (6.0–9.9) | 7.6 (1.3) | 7.2 (5.9–9.9) | **0.006** | **0.047** | **0.012** | 1.00 |
| Malic acid | 8.1 (1.5) | 8.0 (5.2–10.4) | 6.6 (1.6) | 6.1 (5.0–9.1) | 6.3 (1.1) | 6.1 (5.0–8.8) | **0.025** | 0.098 | 0.056 | 1.00 |
| Succinic acid | 8.0 (5.3) | 7.5 (5.3–10.1) | 5.3 (4.1) | 7.3 (0.0–10.3) | 7.4 (1.6) | 7.4 (5.9–10.4) | 0.59 | – | – | – |
| *cis*-Aconitic acid | 9.4 (1.4) | 9.6 (7.4–12.3) | 7.2 (1.3) | 7.0 (5.8–9.3) | 7.2 (1.3) | 6.9 (5.6–9.6) | **0.001** | **0.007** | **0.007** | 1.00 |
| Glutamine | 606.5 (102.2) | 582.0 (472.6–843.8) | 559.4 (80.0) | 572.1 (437.2–647.3) | 576.7 (63.7) | 569.5 (499.0–704.1) | 0.65 | – | – | – |
| Glutamic acid | 51.7 (36.6) | 40.4 (21.1–132.8) | 35.8 (24.1) | 27.1 (13.6–86.1) | 24.6 (7.2) | 22.5 (17.8–37.2) | 0.080 | – | – | – |
| Arginine | 62.6 (19.4) | 63.4 (18.2–92.7) | 68.7 (14.9) | 72.9 (49.0–92.5) | 88.8 (24.1) | 82.4 (59.2–134.9) | **0.033** | 1.00 | **0.029** | 0.22 |
| Citrulline | 27.8 (6.5) | 28.1 (18.2–40.0) | 26.3 (3.6) | 25.2 (21.8–31.5) | 32.9 (4.9) | 33.8 (23.6–40.2) | **0.040** | 1.00 | 0.11 | 0.051 |
| Ornithine | 57.6 (19.5) | 56.5 (34.5–109.7) | 52.6 (14.5) | 52.4 (26.1–69.7) | 45.2 (9.7) | 41.1 (35.2–62.7) | 0.26 | – | – | – |
| Aspartic acid | 3.5 (2.5) | 2.6 (2.1–3.8) | 3.1 (2.3) | 2.2 (1.6–8.9) | 2.3 (0.54) | 1.1 (1.5–3.1) | 0.39 | – | – | – |
| Creatine | 33.0 (20.3) | 30.3 (13.6–88.2) | 37.7 (16.6) | 41.7 (10.9–57.7) | 38.8 (16.9) | 34.9 (20.2–64.9) | 0.56 | – | – | – |
| Creatinine | 57.6 (8.3) | 58.0 (43.4–72.6) | 55.9 (11.7) | 54.8 (40.4–72.2) | 58.6 (12.3) | 58.2 (41.9–78.8) | 0.83 | – | – | – |
| Lactic acid/ Pyruvic acid | 12.0 (2.9) | 11.6 (9.0–20.4) | 11.4 (4.2) | 10.4 (8.5–21.2) | 12.1 (4.3) | 10.4 (7.4–20.1) | 0.43 | – | – | – |
| Pyruvic acid/ Isocitric acid | 11.3 (4.1) | 12.1 (0.0–18.2) | 14.8 (4.4) | 14.8 (5.8–21.2) | 11.4 (4.4) | 12.5 (5.4–17.1) | 0.10 | – | – | – |
| Ornithine/ Citrulline | 2.2 (0.84) | 2.0 (1.1–4.5) | 2.0 (0.58) | 1.8 (1.6–2.6) | 1.4 (0.23) | 1.3 (1.2–1.8) | **0.010** | 1.00 | **0.004** | **0.016** |

* Differences in the absolute concentrations of the major metabolites were assessed with the two-tailed Kruskal–Wallis test.
† Dunn–Bonferroni post hoc method following a significant Kruskal–Wallis test.

**Table 4**
Independent predictors in plasma samples of Fontan patients by logistic regression

| Parameter | Model 1 | | Model 2 | |
|---|---|---|---|---|
| | OR (95% CI) | *P*-value | OR (95% CI) | *P*-value |
| 2-Oxoglutaaric acid | 1.98 (1.05–3.76) | 0.036 | 1.97 (0.98–3.93) | 0.056 |
| *cis*-Aconitic acid | 2.69 (1.04–6.99) | 0.042 | 3.88 (0.99–15.2) | 0.051 |

OR, odds ratio; CI, confidence interval .
Model 1: multiple regression model with stepwise selection-elimination method among metabolites of lactic acid, *cis*-aconitic acid, isocitric acid, 2-oxoglutaric acid, malic acid, arginine, citrulline, and ornithine/citrulline.
Model 2: adjusted for the final selected variables in model 1 along with age and gender.

## 6. Conclusion

In conclusion, the concentrations of organic acids related to the TCA cycle were remarkably associated with the characterization of Fontan patients from controls. Metabolomic profiling may provide important insights into Fontan patient pathophysiology. Validation studies are necessary to ascertain whether our findings can be biomarkers for the early prediction of "silent" Fontan pathophysiologies, which may cause multiple organ dysfunction and diminish the long-term prognosis of the Fontan circulation. Further metabolomic studies are expected to shed light on new targets for diagnosis and therapeutic intervention.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijcha.2021.100921.

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