Original Article

Free Carnitine Levels During Cardiac Peri-Operative Periods with Cardiopulmonary Bypass in Pediatric Patients with Congenital Heart Diseases—Secondary Publication

Yuka Tanabe, MD[1)], Kenji Yamada, MD, PhD[1)], Shigeki Nakashima, MD[1)], Kenji Yasuda, MD, PhD[1)], Maiko Tachi, MD[2)], Yoshifumi Fujimoto, MD[2)], Hironori Kobayashi, MD, PhD[1)], and Takeshi Taketani, MD, PhD[1)]

[1)] Department of Pediatrics, Shimane University Faculty of Medicine, Shimane, Japan
[2)] Department of Cardiovascular Surgery, Shimane University Faculty of Medicine, Shimane, Japan

Objective: Carnitine is essential for long-chain fatty acid metabolism in order to produce biological energy via the mitochondria. Carnitine deficiency can result in cardiomyopathy, hypoglycemia, and other complications. This study aimed to determine how plasma carnitine levels changed before and after open-heart surgery with cardiopulmonary bypass (CPB) in children with congenital heart disease.
Methods: We measured free carnitine (C0) levels in the blood before and after CPB, as well as on each day 7 days after the procedure.
Results: We treated 50 patients with average age of 35 ± 49 months. We identified 19 patients with extracardiac complications, including eight with chromosomal disorder, three with suspected chromosomal disorder, four preterm births, two with digestive disorders, and two with other complications. C0 levels immediately before CPB were 55.8 ± 24.4 nmol/mL, whereas immediately after CPB were significantly lower [33.5 ± 12.9 nmol/mL (the ratio of C0 level before and after CPB were: 64 ± 19%, $p < 0.01$)]. Although there was no significant difference in aortic clamp time, CPB time, or pre-operative blood data, the presence of extracardiac complications was significantly correlated to the C0 level just after CPB (odds ratio, OR: 3.385, 95% confidence interval, CI: 1.858–3.385, $p < 0.01$).
Conclusions: C0 dropped temporarily after CPB and then returned three days later. Since the decrease in C0 after CPB was linked to extracardiac complications in children with congenital heart disease, monitoring pre- and post-operative C0 concentration may be warranted in these patients.

Keywords: free carnitine, acylcarnitine, carnitine deficiency, cardiopulmonary bypass, congenital heart disease

Introduction

Carnitine is essential for generating energy in the mitochondria of either myocardial or skeletal muscle cells. Its deficiency can cause cardiomyopathy and arrhythmia. Some investigators recently pay attention to serum carnitine levels in the perioperative management of cardiac surgery,[1, 2)] although the number of such studies remains small. In patients with congenital heart diseases undergoing surgery, particularly using cardiopulmonary bypass (CPB), the influence of changes in the substance has rarely been considered.

Carnitine exists in the human body as free carnitine (C0) and acylcarnitine (AC). 98% of carnitine is found in the myocardium and the skeletal muscles. Long-chain fatty acids bind to C0, being transported as AC into the

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Corresponding author: Yuka Tanabe, MD, Department of Pediatrics, Shimane University Faculty of Medicine, 89–1 Enya-cho, Izumo City, Shimane 693–8501, Japan
E-mail: yuka.ta.v@gmail.com
Yuka Tanabe (https://orcid.org/0000-0002-7925-1296)
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mitochondria to generate energy.[3, 4] The long-chain fatty acids in the mitochondria provide large amounts of energy via the β-oxidation pathway. The myocardium is dependent on fatty acid β-oxidation for 60–70% of its energy requirements.[5] Lack of C0 prevents fatty acids from entering the mitochondria, impairing fatty acid β-oxidation. As a result, cardiomyocytes develop a severe energy deficiency, causing cardiac symptoms such as cardiomyopathy and arrhythmia.[6] Additionally, carnitine deficiency is known to cause multi-organ symptoms, including hypoglycemia, liver dysfunction, and myalgia/muscle weakeness.[7, 8] Meanwhile, AC accumulation causes comparative deficiency of C0; an elevated AC/C0 ratio is an indicator of carnitine deficiency in the myocardium.[9]

In adults, approximately 75% of carnitine is provided through a daily diet, and the remaining 25% synthesized within the body.[10] In children, however, carnitine synthesis is undeveloped, and the maintenance of the level of the substance is dependent on dietary provision, meaning that its deficiency is liable to occur because of malnutrition, long-term tube feeding without carnitine, and/or an unbalanced diet. Furthermore, secondary carnitine deficiency can be seen after treatment with pivalate-conjugated antibiotics or valproic acid, and in lysinuric protein intolerance or Fanconi syndrome. As for primary deficiency, congenital carnitine transporter (OCTN2) defects is known.[7, 11] C0 is also removed by treatment with CPB or dialysis, which could cause secondary carnitine deficiency.[12, 13] Since carnitine synthetic capacity is immature in children as mentioned above, it is expected that the C0 level drops more significantly in infants than in adults during CPB. In this respect, few studies have addressed carnitine kinetics in the perioperative management of pediatric cardiac surgery using CPB.

We measured the serum level of C0 and the AC/C0 ratio during the perioperative period in children with congenital heart disease undergoing cardiac surgery on CPB to investigate clinical factors associated with decreased carnitine levels.

Methods

Subjects and Study Design

Study participants were pediatric patients who underwent cardiac surgery using CPB for congenital heart disease at Shimane University Hospital between January 2014 and December 2015. This was a single-center observational study that primarily investigated perioperative serum C0 level and AC/C0 ratio. If an individual patient underwent two or more surgeries during the study period, each surgery was regarded as a solitary case.

This study was approved by the Ethics Committee of Shimane University Faculty of Medicine, and informed consent was obtained from the patients or their parents (#20131227-8).

Sample Collection

Venous blood samples were collected for measuring the serum C0 level and the AC/C0 ratio approximately 10 minutes before the start of CPB, 5 minutes after the end of CPB, and on each of the consecutive 7 postoperative days, in principle before breakfast. Blood collection on the postoperative days was conducted simultaneously with clinically necessary blood tests; no specific day or time was additionally designed for blood sample collection.

C0 Level and Total AC Measurements

AC analysis was performed by tandem mass spectrometry (MS/MS), as previously reported, and C0 levels and individual AC values were measured.[14] The reference value used for the C0 level was the normal range (20–60 nmol/mL) used in our institution. The total AC value was approximated by summing individual AC values, because this was not an output of the analysis method used in this study. According to previous studies, the reference value for the AC/C0 ratio was <0.4.[9]

Clinical Information, Including Biochemical Blood Data and Blood and Central Venous Pressures

We collected the following clinical data: age (months), sex, primary disorder, comorbidities, CPB time, aortic cross-clamp time, and operative time. In order to evaluate the preoperative nutritional status, we also collected data on height, weight, body mass index (BMI), and serum levels of total protein, total cholesterol, and triglycerides. Furthermore, we investigated the following: preoperative brain natriuretic peptide (BNP) and human atrial natriuretic peptide (hANP) levels, preoperative and postoperative arterial pressure and central venous pressure (CVP), serum creatine kinase (CK) and creatine kinase-myocardial band (CK-MB) levels,

and CK-MB/CK ratio. These were measured from the preoperative phase to the 7th postoperative day (POD) to evaluate the hemodynamic status, cardiac function, and myocardial damage, except for preoperative CK-MB level which was not measured in all patients. Reference values of the above-mentioned biochemical data were based on patients' age.[15)]

Investigation of Cases with Carnitine Deficiency

Each patient whose preoperative or postoperative C0 level was <20 nmol/mL was investigated for various factors including extracardiac comorbidities, surgical procedures they underwent, and preoperative blood biochemistry data. Clinical indicators were compared between patients in the "low-carnitine" and "normal-carnitine" groups.

Statistical Analysis

Measured values are expressed as a mean $\pm$ standard deviation (*SD*). Changes in perioperative serum C0 levels and AC/C0 ratios were firstly tested by analysis of variance (ANOVA). The C0 level and the AC/C0 ratio at the immediate preoperative phase (pre-CPB) were compared with their immediate postoperative (post-CPB) values using the Wilcoxon signed-rank test. Correlation coefficients were calculated to investigate the association between post-CPB C0 and CK levels (including serum CK-MB concentration and the CK-MB/CK ratio when available), CPB time, aortic cross-clamp time, or operative time. Comparisons between the low-carnitine and normal-carnitine groups were carried out using Fisher's exact test and Wilcoxon's rank-sum test.

Data analysis was conducted with Microsoft Office Excel 2013 (Seattle, WA, USA) and JMP® 16 (Cary, NC, USA), with $p<0.05$ regarded as statistically significant.

Results

Clinical Presentation

Consent was obtained from 48 patients, 24 boys and 24 girls, during the study period. One boy and one girl underwent surgery twice during the study period, resulted in a total of 50 cases. Table 1 shows a summary of patient characteristics. The mean age was 35 ± 49 months. Their primary cardiac diseases were; ventricular septal defect (23 patients), atrial septal defect (9 patients), tetralogy of Fallot (6 patients), truncus arteriosus communis (2 patients), and one patient each for atrioventricular septal defect, cor triatriatum, double-outlet right ventricle, pulmonary atresia with ventricular septal defect, pulmonary artery stenosis, right isomerism, left isomerism, and anomalous origin of the left coronary artery. The latter 2 patients underwent surgery twice. Extracardiac comorbidities were present in 19, comprising chromosomal abnormalities (8 patients; 7 with trisomy 21 and one with abnormal chromosome 1), clinically suspected genetic abnormality (3 patients; one with suspected Noonan syndrome and 2 with suspected Holt–Oram syndrome), premature birth (4 patients), gastrointestinal disorders (2 patients; one with anal atresia surgically corrected and the other

Table 1 Patients' characteristics (n = 50)

Main diagnosis	
VSD	23
ASD	9
TOF	6
ALCAPA	2
Left isomerism	2
TAC	2
AVSD	1
Cor	1
DORV	1
PA/VSD	1
PS	1
Right isomerism	1
Age (months)	35 ± 49
Male gender (%)	25 (50%)
Height (cm)	81.3 ± 27.8
Body weight (kg)	11.8 ± 11.3
BMI (kg/m^2)	15.1 ± 2.1
Total protein (g/dL)	6.7 ± 0.6
Albumin (g/dL)	4.5 ± 0.3
Total cholesterol (mg/dL)	155 ± 30
Triglyceride (mg/dL)	115 ± 61
BNP (pg/mL)	62 ± 72
hANP (pg/mL)	134 ± 135 (n = 38)
Blood pressure before CPB (mmHg)	
Systolic	77 ± 10
Diastolic	41 ± 7
CVP	5 ± 3
Blood pressure after CPB (mmHg)	
Systolic	96 ± 17
Diastolic	51 ± 8
CVP	7 ± 3
CPB time (min.)	157 ± 85
Aortic cross-clamp time (min.)	67 ± 48
Operation time (min.)	292 ± 129

ALCAPA, anomalous left coronary artery arising from the pulmonary artery; ASD, atrial septal defect; AVSD, atrioventricular septal defect; BMI, body mass index; BNP, brain natriuretic peptide; Cor, cor triatriatum; CPB, cardiopulmonary bypass; CVP, central venous pressure; DORV, double outlet right ventricle; hANP, human atrial natriuretic peptide; PA/VSD, pulmonary atresia with ventricular septal defect; PS, pulmonary stenosis; TAC, truncus arteriosus communis; TOF, tetralogy of Fallot; VSD, ventricular septal defect.

Table 2 Serum free carnitine levels and acylcarnitine to free carnitine ratio post cardiopulmonary bypass (CPB) versus pre-CPB

	Pre-CPB	Post-CPB	1 POD	2 POD	3 POD	4 POD	5 POD	6 POD	7 POD
C0 (n) [nmol/mL]	55.8±24.4 (50)	33.5±12.9 (50)**	96.3±42.1 (49)**	67.5±29.4 (38)**	57.6±22.2 (28)	59.7±33.1 (5)	52.6±22.9 (25)	66.5±46.5 (8)	43.2±10.5 (5)
AC/C0 ratio (n)	0.40±0.19 (50)	0.74±0.35 (50)**	0.46±0.26 (49)	0.53±0.29 (38)	0.46±0.37 (28)	0.54±0.76 (5)	0.49±0.42 (25)	0.40±0.44 (8)	0.51±0.28 (5)

**, $p<0.01$. AC/C0, acylcarnitine to free carnitine; C0, free carnitine; POD, postoperative day; n, number of collected samples.

with esophageal stenosis), spina bifida with hypospadias (one patient), and laryngomalacia (one patient). Of the 8 patients with chromosomal abnormality, three also exhibited other comorbidities, including anal atresia, Hirschsprung's disease, and myelodysplastic syndrome. No patient had suspected inherited metabolic diseases including primary carnitine deficiency.

Perioperative Changes in Serum C0 Level and the AC/C0 Ratio

As shown in Table 2, the mean level of serum C0 decreased significantly from pre-CPB 55.8±24.4 nmol/mL to a post-CPB value of 33.5±12.9 nmol/mL (64%±19% of the pre-CPB C0 level, $p<0.01$). During this period of time, none of the patients exhibited hypoglycemia or any other features of typical clinical presentation suggestive of carnitine deficiency. The value significantly increased on the 1st POD to 96.3±42.1 nmol/mL (190%±72% of the pre-CPB C0 level, $p<0.01$), and significantly came down to 67.5±29.4 nmol/mL (138%±48% of the pre-CPB C0 level, $p<0.01$) on the 2nd POD. Thereafter, the mean level of serum C0 exhibited no significant differences compared with the pre-CPB level; 57.6±22.2 nmol/mL (108%±41% of the pre-CPB C0 level) on the 3rd POD, 59.7±33.1 nmol/mL (95%±46%) on the 4th POD, 52.6±22.9 nmol/mL (99%±41%) on the 5th POD, 66.5±46.5 nmol/mL (120%±65%) on the 6th POD, and 43.2±10.5 nmol/mL (124%±33%) on the 7th POD, respectively.

Similarly, the AC/C0 ratio increased markedly from the pre-CPB 0.40±0.19 to 0.74±0.35 immediately after CPB ($p<0.01$) (Table 2). The ratio was settled through the postoperative days; 0.46±0.26 (1st POD), 0.53±0.29 (2nd POD), 0.46±0.37 (3rd POD), 0.54±0.76 (4th POD), 0.49±0.42 (5th POD), 0.40±0.44 (6th POD), and 0.51±0.28 (7th POD), with no significant differences compared with the pre-CPB value.

Perioperative Changes in Serum CK and the CK-MB/CK Ratio

The mean level of serum CK increased from pre-CPB 107±58 U/L to 775±493 U/L immediately after CPB, and peaked on the 1st POD (1,156±2,349 U/L). It subsequently remained elevated until the 4th POD, then returned to the preoperative level after the 5th POD. The CK-MB/CK ratio, an indicator of myocardial damage, rose immediately after CPB to15%±4%, decreased to 5%±2% on the 1st POD, and remained below 6% afterwards. The CK-MB/CK ratio was 6% or greater immediately after CPB in 47/49 cases (94%), and this proportion decreased to 14/38 cases (37%) on the 1st POD. Beyond the period, the CK-MB/CK ratio remained 6% or greater in only one or even none.

Correlations between Post-CPB C0 Level and CPB Time, Aortic Cross-Clamp Time, Operative Time, or Post-CPB CK Level

In terms of the associations of the post-CPB C0 level to the clinical parameters, a correlation coefficient with CPB time (a mean 157±85 min) was 0.22 ($p=0.12$), that with the aortic cross-clamp time (67±48 min) 0.03 ($p=0.84$), and that with total operative time (292±129 min) 0.21 ($p=0.15$).

Correlation coefficients between the post-CPB C0 level and the post-CPB CK level, CK-MB level, or CK-MB/CK ratio were 0.07 ($p=0.62$), <0.01 ($p=0.98$), and 0.24 ($p=0.14$), respectively.

Patients in Whom C0 Level Was Below the Reference Value

In this study, the C0 level was below 20 nmol/mL after CPB in 5 occasions and below the reference value prior to CPB in one (Table 3). Among these 6 occasions, two instances refer to the same patient (patient 5), who underwent surgery twice during the study period.

Patient 1, a seven-month-old boy with atrial septal defect, had been born with an extremely low body

Table 3 Clinical information of five patients with carnitine deficiency after CPB

Patient number	Age	Main diagnosis	Extracardiac complication	Details of surgery	Free carnitine levels (nmol/mL)/AC-C0 ratio				TP (g/dL)	Albumin (g/dL)	T-Cho (mg/dL)	TG (mg/dL)	BNP (pg/mL)	CK (U/L)
					Pre-CPB	Post-CPB	1 POD	2 POD						pre/post CPB
1	7m	ASD	ELBWI was fed carnitine-free formula	ASD direct closure, PDA ligation	29.0/**0.45**	**16.0/0.69**	**119.0**/0.18	**61.7**/0.14	6.2	4.1	**102**	54	**183.0**	91/**766**
2	1y2m	TOF	Trisomy-21, after surgery for anal atresia	VSD patch closure, RVOTR, PV commissurotomy, tethering release, mPA posterior wall plasty, mPA patch augmentation, TVP	27.1/**0.44**	**14.5/1.20**	**83.1/0.44**	**72.2/0.51**	6.2	4.2	137	80	**53.0**	223/**1,580**
3	2m	VSD	Trisomy-21, after surgery for Hirschsprung's disease	VSD patch closure, mVSD4 direct closure, ASD direct closure, PDA ligament	20.6/**0.81**	**14.7/1.37**	54.5/0.30	52.5/**0.40**	5.7	3.8	131	87	**293.6**	123/**1,489**
4	1y3m	TOF	Laryngomalacia	Valve sparing method: VSD patch closure, RVOTR, PV commissurotomy, tethering repair, mPA patch augmentation, PDA division	42.8/0.29	**18.0/0.98**	**64.2**/0.38	40.5/**1.51**	7.4	4.7	181	**186**	**31.8**	120/**1,759**
5	9m	Left isomerism	Several surgeries were performed before participation in this study	TCPS, CAVVP, RF ablation, mPA division, PV closure, PAP (patch augmentation)	25.4/**0.88**	**15.8/1.29**	35.5/**0.75**	47.7/**0.64**	6.4	4.4	138	68	**145.0**	22/**747**
5	1y9m			TCPC, HV rerouting	**17.8**/0.33	21.5/**0.57**	**64.6**/0.30	32.8/**0.86**	6.8	4.3	139	**210**	**26.0**	289/270

Patient 5 underwent two surgeries at the age of 9 months and 1 year 9 months. Abnormal date is in bold and underlined. AC-C0, acylcarnitine/free carnitine; ASD, atrial septal defect; BNP, brain natriuretic peptide; CAVVP, common atrioventricular valve plasty; CK, creatine kinase; CPB, cardiopulmonary bypass; ELBWI, extremely low birth weight infant; HV, hepatic vein; m, month(s); mPA, main pulmonary artery; mVSD, muscular ventricular septal defect; PAP, pulmonary artery plasty; PDA, patent ductus arteriosus; POD, postoperative day, PV, pulmonary valve; RF, radiofrequency; RVOTR, right ventricular outflow tract reconstruction; TCPC, total cavopulmonary connection; TCPS, total cavopulmonary shunt; TG, triglyceride; TOF, tetralogy of Fallot; TP, total protein; TVP, tricuspid valve plasty; T-Cho, total cholesterol; y, year(s).

weight and had been tube fed with a formula that did not contain carnitine. His pre-CPB C0 level was in the lower range of normal (29.0 nmol/mL), and his pre-CPB AC/C0 ratio was high (0.45). Postoperatively, his C0 level dropped to 16.0 nmol/mL.

Patient 2 was a 14-month-old boy with tetralogy of Fallot in the setting of trisomy 21 and had undergone surgery for anal atresia before participating this study. His C0 level decreased from preoperative 27.1 nmol/mL to postoperative 14.5 nmol/mL.

Patient 3 was a two-month-old boy with ventricular septal defect, trisomy 21, and had undergone surgery for Hirschsprung's disease. His preoperative C0 level was in the lower range of normal (20.6 nmol/mL), and the AC/C0 ratio was high (0.81), whereas his postoperative C0 level became 14.7 nmol/mL and the AC/C0 ratio 1.37.

Patient 4 was a boy aged 15 months with tetralogy of Fallot and laryngomalacia. His preoperative C0 level and AC/C0 ratio were 42.8 nmol/mL and 0.29, respectively, and the postoperative values were 18.0 nmol/mL and 0.98, respectively. The C0 levels of these four patients were above the reference levels after the 1st POD.

Patient 5 presented a specific clinical course and had already undergone several surgical procedures before participating our study. During the study, he underwent cardiac surgery twice at the ages of 9 and 21 months. At the time of the former procedure (age 9 months), the C0 level decreased from preoperative 24.5 nmol/mL to postoperative 15.8 nmol/mL; then increased to 35.5 nmol/mL on the 1st POD, but dropped again to 15.8 nmol/mL on the 6th POD. This was the only occasion in which the C0 level fell below the reference value throughout the perioperative period. At the time of the latter surgery (age 21 months), the preoperative C0 level was low (17.8 nmol/mL). The level rose above the reference value immediately after CPB and remained over 20 nmol/mL thereafter. The AC/C0 ratio in Patient 5 changed from preoperative 0.88 to postoperative 1.29 after the former surgery and from 0.33 to 0.57 following the latter surgery. Preoperative malnutrition such as hypoalbuminemia or hypolipidemia was obvious in none of these six occasions.

These 6 cases were defined as a low-carnitine group in which C0 was below the reference level either preoperatively or postoperatively, and compared with the other 44 cases in which C0 was within a normal range (a normal-carnitine group). There were significant dif-

Table 4 Comparison between the low C0 and the normal C0 groups after CPB

Characteristics	Low C0 (n=6)	Normal C0 (n=44)	*p* value
Extracardiac complication (%)	6 (100)	13 (30)	<0.01
Age (months)	11±7	38±52	ns
Male gender (%)	6 (100)	19 (43)	<0.05
Height (cm)	65.3±10.1	83.6±28.7	ns
Body weight (kg)	6.5±2.6	12.5±11.8	ns
BMI (kg/m^2)	14.5±1.8	15.1±2.2	ns
Pre CPB			
C0 (nmol/mL)	27.1±8.7	59.7±23.3	<0.01
AC/C0 ratio	0.53±0.25	0.38±0.18	ns
Total protein (g/dL)	6.5±0.6	6.8±0.6	ns
Albumin (g/dL)	4.3±0.3	4.5±0.3	ns
Total cholesterol (mg/dL)	138±25	158±30	ns
Triglyceride (mg/dL)	114±66	115±61	ns
BNP (pg/mL)	122±106	53±63	<0.05
Blood pressure before surgery (mmHg)			
Systolic	74±7	78±11	ns
Diastolic	42±6	41±7	ns
CVP	7±3	5±2	ns
Blood pressure after surgery (mmHg)			
Systolic	83±11	98±17	<0.05
Diastolic	49±5	52±8	ns
CVP	10±3	6±3	<0.01
CPB time (min.)	198±94	151±83	ns
Aortic cross-clamp time (min.)	82±60	64±47	ns
Operation time (min.)	349±134	284±128	ns

AC/C0, acylcarnitine to free carnitine; BMI, body mass index; BNP, brain natriuretic peptide; CPB, cardiopulmonary bypass; CVP, central venous pressure; C0, free carnitine; ns, not significant.

ferences between these groups in the presence of extracardiac comorbidities, sex, pre-CPB C0 level, pre-CPB BNP, post-CPB systolic pressure, and post-CPB CVP (Table 4). Post-CPB systolic pressure and CVP were strongly affected by age. When these two factors were adjusted for age, only post-CPB CVP exhibited a significant difference. Furthermore, extracardiac comorbidities were present in all patients with low C0 levels (the low-carnitine group); association was statistically significant between the presence of an extracardiac complication and a low C0 level (odds ratio [OR], 3.385; 95% confidence interval [95% CI], 1.858–3.385; $p<0.01$).

Discussion

In this study, we demonstrated perioperative changes in the serum C0 level and the AC/C0 ratio in children with congenital heart diseases who underwent cardiac surgery using CPB. Specifically, the C0 level decreased significantly soon after CPB compared with before going on CPB, then rose to a peak value on the first day after surgery, gradually decreased on the 2nd POD to a value that was still significantly higher than the preoperative level, and returned to the preoperative level on the 3rd POD. Nemoto et al. reported that CPB removed C0 in adult patients.[12] Our results reconfirmed their findings in pediatric patients. In the same report, serum C0 levels in adults undergoing open heart surgery did not recover by two hours after off CPB; subsequent changes were not investigated.[12] Our results revealed that, in children, the postoperative C0 level typically recovered within one day, even higher than the preoperative level on the 1st POD. Unfortunately, we did not track the postoperative C0 level on an hourly basis. It remains unknown how long the C0 level stayed low, how low it actually became, or when was exactly the timing of the lowest C0 level.

We also found that the AC/C0 ratio was already high preoperatively and was elevated further after CPB, indicating relative deficiency of C0 in the myocardium. In the present study, the AC/C0 ratio was ≥0.4 before CPB in 22 of 50 cases (44%). As far as the reference value for the AC/C0 ratio in children was similar to that in adults,[16] our results suggested that infants with congenital heart diseases could have relative carnitine deficiency in the myocardium even before surgery. On the other hand, the elevated AC/C0 ratio at the post-CPB might not reflect relative carnitine deficiency. The C0 is removed by CPB, but the AC stays.[13] Thus, the AC/C0 ratio superficially tends to rise after CPB regardless of the carnitine level.

As described above, infants with congenital heart diseases have an overall tendency of carnitine deficiency; furthermore, our results suggest that particular attention to carnitine deficiency is required for children with extracardiac comorbidities. In this study, extracardiac comorbidities were present in all six cases of the low-carnitine group. In the five occasions with low C0 level or a lower limit of the normal range preoperatively, their carnitine intake and absorption were considered inadequate due to gastrointestinal disorders and/or previous surgery. The preoperative BNP level was significantly higher in the low-carnitine group, suggesting that children with poor general conditions are more susceptible to perioperative carnitine deficiency. Such poor conditions include not only malnutrition but also cardiac failure. Our results also showed that carnitine deficiency cannot be estimated from general biochemical data, such as total protein and total cholesterol levels. Because carnitine deficiency may be present even when malnutrition is not biochemically detected, carnitine monitoring should be warranted for children with extracardiac comorbidities and those who have undergone or are scheduled to undergo multiple surgeries.

It remains unclear, in our study, to what extent the transient decline in C0 and relative carnitine deficiency in the tissues affect cardiac and other organs' functions. Previous reports showed that carnitine deficiency lasting only a few days could cause generalized seizures, unconsciousness, encephalopathy, and cardiomyopathy due to severe hypoglycemia,[10, 17] but, to the best of our knowledge, no study has reported clinical symptoms caused by a low C0 level lasting for less than one day. In the present study, no symptoms suggestive of carnitine deficiency were observed in any of our cases, including the six occasions of the low-carnitine group. This indicates that the transiently low level of carnitine is unlikely a major clinical issue. We also found no association between postoperative low C0 and biochemical data (such as CK and the CK-MB/CK ratio) which reflect myocardial damage. suggesting that transient carnitine deficiency does not cause the degree of myocardial damage which affects serum CK concentration. Meanwhile, a temporarily low carnitine level might influence circulatory hemodynamics even in the absence of evident clinical signs, because the post-CPB CVP was consid-

erably higher in the low-carnitine group than in the normal-carnitine group. The direct causal relationship, nonetheless, is unclear between a low carnitine level and elevated CVP; CVP is readily affected by other factors such as invasive surgical procedures and transfusions.

A previous report showed that carnitine deficiency was caused by hemodialysis impaired myocardial fatty acid oxidation.[18] Others described that carnitine supplementation was effective for maintaining cardiac function and preventing arrhythmia in adults undergoing coronary artery bypass surgery.[19, 20] In contrast, some studies reported that perioperative carnitine supplementation was clinically meaningless.[21, 22] Our results suggest that differential diagnosis is necessary for carnitine deficiency in some cases, although none of our patients required carnitine supplementation. Specifically, younger patients with complex cardiac anomalies requiring multiple surgeries seem at risk of developing a clinically significant deficiency of carnitine. This is because such infants are more likely exposed to hypercatabolism due to the invasive nature of their surgery on top of diet restriction during the perioperative period. A study focusing solely on such patients would draw a conclusion whether perioperative supplementation of carnitine should work or not.

In this study, we did not find any association between the decrease in the C0 level immediately after CPB and the CPB time, aortic cross-clamp time, or operative time. The serum C0 level does not necessarily reflect C0 concentration within the tissues.[23] We initially anticipated that a longer extracorporeal circulation time or operative time should reduce the C0 level with carnitine depleted and its supply interrupted. This was not the case. Intraoperative damage to the myocardial tissue could have released carnitine into the bloodstream, maintaining the serum C0 level. This hypothesis can also explain why the C0 level was elevated on the first day after surgery. The fact that there was no clear association between the CK and the C0 concentrations on the first POD contravenes our hypothesis.

Finally, this study has some limitations. First, the sample size was small for determining changes in the C0 level and the AC/C0 ratio after the 4th POD, because we had not specified a blood collection protocol. In addition, the low-carnitine group is comprised of only six cases; we were unable to conclude statistical association between carnitine deficiency and circulatory hemodynamics. We believe that, with a sufficient sample size, significant associations would be identified between the C0 level and some other factors, such as the operative time (including CPB time and aortic cross-clamp time) and age. These were not proven this time. In fact, being in low age might be an inherent risk factor for carnitine deficiency, because of a undeveloped state of carnitine biosynthesis. The quantitative analysis of cardiac function by echocardiography is difficult during open heart surgery and in the open chest environment immediately after surgery, because the acoustic window is poor. Furthermore, parameters on echocardiography (for example changes in ejection fraction) are markedly affected by the surgical procedures; therefore, evaluating their associations with the postoperative change in carnitine was difficult. In addition, we did not conduct comparisons with cases in which CPB was not used. It was undetermined, therefore, whether factors other than CPB, such as surgical insults themselves, would affect the carnitine level. Further studies are required to investigate the effects of those factors such as fasting and damage to the muscles or tissues other than the heart.

Conclusions

Serum the C0 level in infants with congenital heart diseases decreased after CPB, and began to recover within one day. Even with normal serum C0 concentration preoperatively, the AC/C0 ratio was elevated approximately in a half of our cases, and relative carnitine deficiency of the myocardium should be noted for infants with congenital heart diseases. Particularly, children with gastrointestinal diseases or other extracardiac comorbidities and those undergoing multiple surgeries are at higher risk of carnitine deficiency. Careful attention should be paid to perioperative carnitine levels, even if carnitine deficiency presented does not cause a major clinical issue.

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Conflicts of Interest

The authors have no conflicts of interest (COI) to disclose with regard to this study.

Author Contributions

Yuka Tanabe contributed to protocol preparation, implementation, data collection, statistical analysis, data analysis, manuscript preparation, and presentation of study results. Kenji Yamada contributed to statistical analysis, data analysis, manuscript preparation of original Japanese draft, presentation of study results, data interpretation, English translation from Japanese draft, and re-editing English manuscript. Hironori Kobayashi contributed to manuscript preparation, manuscript structure, presentation of study results, and data interpretation. Shigeki Nakashima contributed to protocol preparation, implementation, and presentation of study results. Maiko Tachi and Yoshifumi Fujimoto contributed to implementation, data collection, and presentation of study results. Kenji Yasuda and Takeshi Taketani contributed to manuscript structure, statistical analysis, data interpretation, and critical review of the content of the paper.

Note

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