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ORIGINAL ARTICLE

Effect of levocarnitine supplementation on myocardial strain in children with acute kidney injury receiving continuous kidney replacement therapy: a pilot study

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Abstract

Background Carnitine plays a key role in energy production in the myocardium and is efficiently removed by continuous kidney replacement therapy (CKRT). Effects of levocarnitine supplementation on myocardial function in children receiving CKRT have not been investigated.

Methods This controlled pilot cohort study of 48 children investigated effects of levocarnitine supplementation on myocardial strain in children receiving CKRT for acute kidney injury (AKI). Children (n = 9) with AKI had total (TC) and free plasma carnitine (FC) measurements and echocardiogram for longitudinal and circumferential strain at baseline (prior to CKRT) and follow-up (on CKRT for ≥ 1 week with intravenous levocarnitine supplementation, 20 mg/kg/day). Intervention group was compared with three controls: (1) CKRT controls (n = 10) received CKRT ≥ 1 week (+AKI, no levocarnitine), (2) ICU controls (n = 9) were parenteral nutrition-dependent for ≥ 1 week (no AKI, no levocarnitine), and (3) healthy controls (n = 20).

Results In the Intervention group, TC and FC increased from 36.0 and 18 μmol/L to 93.5 and 74.5 μmol/L after supplementation. TC and FC of unsupplemented CKRT controls declined from 27.2 and 18.6 μmol/L to 12.4 and 6.6 μmol/L, which was lower vs. ICU controls (TC 32.0, FC 26.0 μmol/L), $p < 0.05$. Longitudinal and circumferential strain of the Intervention group improved from − 18.5% and − 18.3% to − 21.1% and − 27.6% after levocarnitine supplementation; strain of CKRT controls (-14.4%, -20%) remained impaired and was lower vs. Intervention and Healthy Control groups at follow-up, $p < 0.05$.

Conclusions Levocarnitine supplementation is associated with repletion of plasma carnitine and improvement in myocardial strain and may benefit pediatric patients undergoing prolonged CKRT.

Keywords Children · Acute kidney injury · Continuous kidney replacement therapy · Carnitine · Cardiovascular · Pediatric · Total parenteral nutrition

Introduction

Carnitine is a naturally occurring amino acid derivative which is essential for transporting fatty acids into the mitochondrial matrix for the production of energy by beta-oxidation, providing the primary source of energy to the myocardium. Carnitine also helps to protect myocytes from oxidative damage and apoptosis by scavenging harmful excess acyl groups and oxidative radicals [1]. Because cardiac muscle cells are unable to synthesize carnitine de novo, it must be acquired exogenously. Dialysis-related carnitine deficiency is known to be common among adult and pediatric chronic hemodialysis (HD) patients, due to the efficient removal of carnitine with each HD treatment [2], resulting in decreased energy production in the myocardium. As such, dialysis-related carnitine deficiency has been linked with adverse cardiovascular effects in adults [3]. Supplementation with intravenous (IV) levocarnitine (carnitine) repletes plasma and muscle carnitine stores and has been associated with improvement in numerous cardiovascular parameters, including improved left ventricular ejection fraction [4–8], left ventricular mass index (LVMI) [5, 8], pulse wave velocity [9], brain natriuretic peptide (BNP) [8], and myocardial fatty acid metabolism [10, 11], as well as

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reduction in hypotension [12–14] and arrhythmias [15, 16] in adult chronic HD patients. As signs of cardiovascular abnormalities may be more subtle in dialyzed children, strain analysis allows for the detection of subclinical cardiac dysfunction that may not be apparent by traditional echocardiography. We previously showed that carnitine supplementation improves myocardial strain in a cohort of pediatric chronic HD patients [17]. Compared with chronic HD, carnitine is even more rapidly depleted by continuous kidney replacement therapy (CKRT), with losses approximating 80% of intake [18]. The effect of carnitine deficiency and supplementation on cardiovascular function in patients receiving CKRT has not been previously investigated.

Therefore, our objective was to conduct a controlled pilot cohort study investigating the effect of carnitine supplementation on myocardial strain in children receiving CKRT. Given the efficient removal of carnitine by CKRT and the essential role of carnitine in providing energy to the myocardium, we hypothesized that carnitine supplementation would improve left ventricular function in children receiving CKRT.

Methods

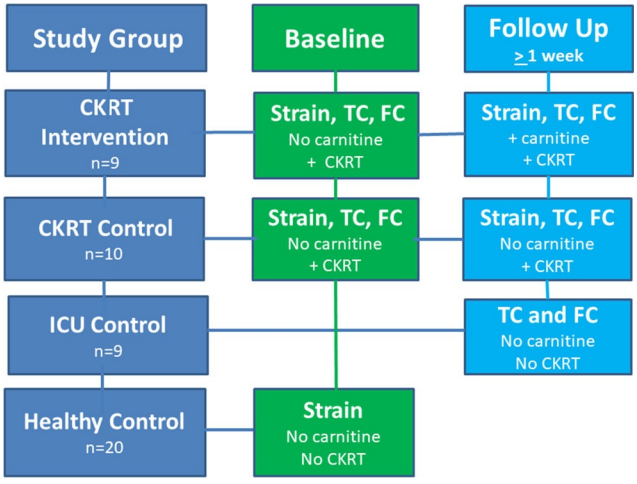
Study design and enrollment

This was a controlled pilot cohort study of children with acute kidney injury (AKI) receiving CKRT, where the exposure of interest was IV carnitine supplementation and the outcomes of interest were plasma carnitine levels and myocardial function as measured by longitudinal and circumferential strain. An overview of the study design is shown in Fig. 1. Children ages 1–21 years with AKI requiring CKRT who were admitted to the pediatric intensive care unit (ICU) at Children's National Hospital from 2015 to 2018 were eligible to prospectively enroll in the "CKRT Intervention group," if they were total parenteral nutrition (TPN)-dependent and not receiving any enteral or IV carnitine prior to enrollment. Patients who were receiving any prior carnitine supplementation, receiving enteral nutrition support, those with stage 5 chronic kidney disease (CKD 5), history of prior acute dialysis, or a primary cardiac diagnosis, were excluded from enrolling in this CKRT Intervention group. Any patient who did not remain on CKRT for at least 1 week was also excluded from the analysis. Parameters of the CKRT Intervention group were compared against three different control groups. Children ages 1–21 years were included in one of the three control groups, according to the following criteria. The "CKRT Control group" included critically ill children who received CKRT for ≥ 1 week but did not receive any carnitine supplementation, were TPN-dependent, and had weekly carnitine levels measured and echocardiograms performed as part of another study at Children's National Hospital between 2011 and 2014. Exclusion criteria for this group were the same as listed above for the CKRT Intervention group. The "ICU Control group" included critically ill children admitted to the pediatric ICU from 2015 to 2018 who were TPN-dependent without carnitine supplementation for ≥ 1 week and did not require CKRT. Children with AKI (stage 3), defined as eGFR < 35 mL/min/1.73 m^2 according to KDIGO AKI criteria for children [19], were excluded from the ICU Control group. Finally, the "Healthy Control group" included healthy children with no history of acute or chronic disease. The healthy controls served primarily as an age-matched reference group for myocardial strain, since presently there are no established normal reference ranges for myocardial strain in children.

The study (NCT01941823) was approved by the institutional review board of Children's National Hospital. Informed consent of parents/participants was obtained, and the study was conducted in accordance with the Declaration of Helsinki.

Plasma carnitine monitoring

CKRT Intervention group and CKRT Control group participants had plasma total and free carnitine levels measured at baseline (prior to start of CKRT) and follow-up (≥ 1 week on CKRT) time points, whereas the ICU Control group had carnitine levels measured at one time point (≥ 1 week on TPN with no carnitine supplementation). Plasma carnitine levels were not measured in the Healthy Control group, since normal reference ranges for carnitine levels in children are already established. Total and free carnitine was analyzed using liquid



An overview of the study design is shown. The parameters measured for each group at baseline are shown in green boxes and those measured at follow-up are shown in light blue boxes. Age-specific normal carnitine levels are already established in healthy children, and therefore were not measured. Continuous Kidney Replacement Therapy (CKRT), Intensive care unit (ICU), TC (total carnitine), FC (free carnitine)

Fig. 1 Study design

chromatography/tandem mass spectrometry (LC/MS/MS) methodology by Quest Research Lab (San Juan Capistrano, CA). Normal total and free carnitine levels were defined according to standard age-specific cut-points as follows: > 35 μmol/L and > 24 μmol/L for ages 1–6 years, > 28 μmol/L and > 22 μmol/L for ages 7–10 years, > 34 μmol/L and > 22 μmol/L for ages 11–17 years, and > 34 μmol/L and > 25 μmol/L for ages 18–21 years old [20].

Carnitine supplementation

All study patients in the "CKRT Intervention group" had IV carnitine added to their TPN at a dose of 20 mg/kg/day. Thus, the daily IV carnitine dose was infused continuously over a 24-h period, as a component of their TPN. None of the patients in the "ICU Control group," the "CKRT Control group," or the "Healthy Control group" received any oral or IV carnitine supplementation.

Echocardiography for strain

Echocardiograms were performed at two time points in the "CKRT Intervention" and "CKRT Control group" participants. The CKRT patients and controls had one echocardiogram performed at baseline, and the second was performed at follow-up (≥ 1 week). In the "Healthy Control group," one echocardiogram was performed at a single outpatient visit. Echocardiograms were not performed on the ICU Control group.

All echocardiograms were performed by a pediatric sonographer using the iE33 xMatrix DS Ultrasound System (Philips North America Corporation, Andover, MA, USA), according to American Society of Echocardiography standards. Myocardial strain analysis by speckle tracking was performed by a single pediatric cardiologist who was blinded to the participants' clinical information using Syngo Velocity Vector Imaging software (Siemens, Germany) on DICOM standard digital echocardiograms. Circumferential and longitudinal strain of the left ventricle was obtained from the parasternal short-axis view and apical 4 chamber view, respectively. Endocardial tracings were performed manually on the parasternal short axis, and apical four chamber images were obtained twice during end systole for each patient, and the average was recorded. Short axis measurements of circumferential and longitudinal strain were recorded. Strain was calculated by measuring the end-systolic distance between two speckles of tracked endocardium minus the original distance, divided by the original distance, and reported as a percentage (%). Because the myocardium contracts in the longitudinal and circumferential directions during systole, strain values are negative percentages, and more negative values indicate better cardiac contractility.

Pediatric Logistic Organ Dysfunction-2 score

Pediatric Logistic Organ Dysfunction-2 (PELOD-2) scores were calculated at baseline and follow-up as an indicator of severity of illness in the CKRT intervention, CKRT Control, and ICU Control groups, according to the previously published method [21]. PELOD-2, a validated tool for assessment of the severity of multiple organ dysfunction syndromes in critically ill children, includes the following indicators: neurologic (Glasgow Coma Score, pupillary reaction), cardiovascular (lactatemia, mean arterial pressure), renal (serum creatinine), respiratory (PaO_2, $PaCO_2$, mechanical ventilation), and hematologic (while blood cell count, platelets). For patients in the CKRT Intervention and CKRT Control groups, the renal score was based on the serum creatinine at initiation of CKRT. For those in the ICU Control group, the renal score was based on serum creatinine on the day of plasma carnitine measurement. Based on these indicators, the PELOD-2 score is determined on a scale of 0 to 33, where score 33 indicates maximum severity of illness.

Statistical analysis

All statistical analyses were conducted using Stata 14.0 (StataCorp LP, College Station, TX, USA). As this was a pilot study with small sample size, multivariable analyses were not performed.

Demographic and clinical characteristics Demographics and clinical characteristics, including age, sex, race, PELOD-2 score, BMI z-score, hemoglobin (g/dL), ICU days prior to initiation of CKRT, days on CKRT at follow-up, and % of patients with sepsis, were compared between the study and control groups. For comparisons between 2 groups for continuous variables, Wilcoxon rank-sum was used. For comparison of 3 or more groups for continuous variables, the Kruskal-Wallis test was used with post hoc testing by Dunn's pairwise comparison test performed only for variables that were significantly different by Kruskal-Wallis ($p < 0.05$). Categorical variables (sex, race, % of patients with sepsis) were compared by Fisher's exact test, with p value < 0.05 considered significantly different.

Plasma carnitine Changes in plasma total and free carnitine from baseline to follow-up were compared by Wilcoxon rank-sum within the CKRT Intervention group and CKRT Control group, respectively. In addition to being compared within groups, plasma total and free carnitine levels were also compared between the CKRT Intervention and CKRT Control groups at each time point (baseline and follow-up) by Wilcoxon rank-sum. Furthermore, the total and free carnitine levels of the CKRT Intervention group and the CKRT Control

group at baseline and follow-up were compared to the carnitine levels of the ICU Control group by Wilcoxon rank-sum.

Myocardial strain Myocardial strain was compared between the CKRT Intervention and the CKRT Control groups at baseline and follow-up by Wilcoxon rank-sum. Finally, myocardial strain of the CKRT Intervention group and the CKRT Control group was compared to myocardial strain of the Healthy Control group by Wilcoxon rank-sum.

Results

Study population

The study population comprised a total of 48 participants: 9 in the CKRT Intervention group, 10 in the CKRT Control group, 9 in the ICU Control group, and 20 in the Healthy Control group. Demographics and clinical characteristics of the study population are summarized in Table 1. The CKRT Intervention group was racially and ethnically diverse (55.6% Caucasian, 11.1% African American, 33.3% other), 66.6% female, and had a median (IQR) age of 10 (5, 15) years and BMI z-score of + 0.4 (0.3, 1.2). There were no statistically significant differences detected in the age, sex, BMI z-score, or race distribution of the CKRT Intervention group as compared to the CKRT Control, ICU Control, or Healthy Control groups. The most common underlying diagnosis among the CKRT Intervention and CKRT Control patients was bone marrow transplant. The severity of illness as determined by PELOD scoring was 12 (IQR 9, 13), on a scale from 0 to 33, in the CKRT Intervention group and 11 (IQR 9, 13) in the CKRT Control group at baseline, $p = 0.91$. The majority of study participants had sepsis, and there was no statistically significant difference detected in the prevalence of sepsis between groups, $p = 0.32$. The median hemoglobin level of the CKRT Control group was higher at follow-up (10.5 g/dL) compared to baseline (9.4 g/dL, $p = 0.02$) and was also higher compared to the CKRT Intervention (9.1 g/dL, $p = 0.02$) and ICU Control groups (9.5 g/dL, $p = 0.03$) at follow-up. We did not detect a statistically significant difference in the total number of days on CKRT at follow-up between the CKRT Intervention group (11 [IQR 10, 14]) and the CKRT Control group (13 [IQR 8, 18]), $p = 0.12$. The length of ICU stay of the ICU Control group at the time of measurement of plasma carnitine was 11 (IQR 10, 12) days ($p = 0.87$ and $p = 0.06$ compared to the number of CKRT days of the CKRT Intervention and CKRT Control groups at follow-up, respectively). The mode of CKRT used for all patients was continuous veno-venous hemodiafiltration (CVVDHF), dose of 2000 mL/1.73 m^2/h, using a Prismaflex dialysis machine (Baxter, USA) with citrate anticoagulation. None of the patients died during the study period.

Plasma carnitine levels

The results of the median total and free carnitine levels of the study and control groups are illustrated in Fig. 2. At baseline, median total carnitine of the CKRT Intervention group (36.0 μmol/L, IQR 11.0, 57.8) and the CKRT Control group (27.2 μmol/L, IQR 24.8, 45.5) did not appear statistically different from the ICU Control group (32.0 μmol/L, IQR 27.5, 46.3, $p = 0.82$). Baseline free carnitine of the CKRT Intervention group (18.5 μmol/L, IQR 5.5, 31.3) and the CKRT Control group (18.6 μmol/L,17.4, 32.8 IQR) were not significantly different from each other ($p = 0.47$) but were lower compared to ICU Controls, 26.0 μmol/L, IQR 21.5, 37.4 ($p = 0.04$).

As expected, median total and free carnitine levels significantly increased in the CKRT Intervention group after carnitine supplementation (total carnitine 93.5 μmol/L, IQR 56.8, 98.6, $p = 0.006$ and free carnitine 74.5 μmol/L, IQR 46.2, 80.8, $p = 0.004$ compared to baseline). Conversely, carnitine levels of the CKRT Control patients decreased, and these patients exhibited severe carnitine deficiency, evidenced by total carnitine (12.4 μmol/L, IQR 10.5, 24.8, $p = 0.004$) and free carnitine (6.6 μmol/L, IQR 6.3, 17.0, $p = 0.002$ compared to baseline). Total and free carnitine levels of patients in the CKRT Control group were significantly lower as compared to both the CKRT Intervention group at follow-up ($p = 0.004$ for both total and free carnitine) and the ICU Control group ($p = 0.001$ for both total and free carnitine).

Myocardial strain

Results of the longitudinal strain analysis are shown in Table 2. At baseline, both the CKRT Intervention and CKRT Control groups had worse longitudinal strain (− 18.5% and − 13.8%) compared to the healthy controls (− 22.6%). The CKRT Intervention group that received IV carnitine supplementation demonstrated improved myocardial contractility over time as evidenced by improvement in longitudinal strain from − 18.5% at baseline to − 21.1% at follow-up, which was similar to that of healthy controls (− 22.6%). Meanwhile the longitudinal strain of the CKRT Control group that did not receive carnitine supplementation remained impaired (− 14.4%) at follow-up, in comparison to both the CKRT Intervention and Healthy Control groups.

Similarly, circumferential strain improved in the patients receiving carnitine supplementation in the CKRT Intervention group, as compared to those who did not, as shown in Table 3. At baseline, both the CKRT Intervention and Control groups had worse circumferential strain (− 18.3% and − 19.0%) compared to the healthy controls (− 25.7%). The CKRT Intervention group, that received IV carnitine supplementation, demonstrated improved myocardial contractility over time in the circumferential direction, evidenced by improvement in circumferential strain from − 18.3% at

Table 1 Demographics and clinical characteristics

	CKRT Intervention group $n = 9$	CKRT Control group $n = 10$	ICU Control group $n = 9$	Healthy Controlgroup $n = 20$	p^b
Age (years)	10 (5, 15)	13 (8, 18)	11 (8, 14)	10 (6, 13)	0.37
Caucasian	55.6%	10%	22.2%	30%	
AA	11.1%	40%	33.3%	50%	0.16
Other	33.3%	50%	44.4%	20%	
Female	66.6%	60%	55.5%	75%	0.70
Underlying diagnoses	BMT (33%) Tumor lysis syndrome (11%) HUS(11%) DIC (11%) Influenza (11%) Duodenal perforation (11%) Necrotizing fasciitis (11%)	BMT (100%)	Bacteremia (22.2%) SBO, SIRS (22.2%) Pneumonia, ARDS (22.2%) FIRES, DIC (11.1%) Pancreatitis (11.1%) Necrotizing fasciitis (11.1%)	NA	NA
PELOD-2 score[a]					
Baseline	12 (9, 13)	11 (9, 13)	NA	NA	0.91
Follow-up	9 (8, 11)	11 (8, 13)	7 (6, 9)		0.07
Sepsis present (% of patients)	100%	70%	77.8%	NA	0.32
BMI z-score	0.4 (0.3, 1.2)	0.7 (− 0.2, 1, 2)	0.5 (0.2, 1.5)	0.4 (− 0.4, 1.1)	0.59
Hemoglobin (g/dL)					
Baseline	8.6 (8.1, 8.9)	9.4 (9, 0, 9, 8)$^{\gamma}$	NA	NA	0.15
Follow-up	9.1 (8.5, 10.1)$^{\alpha}$	10.5 (10, 0, 12.4)$^{\alpha\beta\gamma}$	9.5 (8.4, 9.9)$^{\beta}$		0.02*
Mode of CKRT					
CVVHFD	100%	100%	NA	NA	1.0
ICU days prior to CKRT	4 (1, 5)	8 (2, 17)	NA	NA	0.23
CKRT days *(at follow-up)*	11 (10,14)	13 (8, 18)	NA	NA	0.12

[a] Pediatric Logistic Organ Dysfunction-2 score on a scale of 0–33, where 33 indicates maximum severity of illness

[b] p values for comparison between groups by Fisher's exact test (categorical variables) or Kruskal-Wallis test (continuous variables), Categorical data are presented as proportion (%) and compared between groups by Fisher's exact test Continuous data are presented as median, IQR. Comparison of continuous variables between 2 groups was done by Wilcoxon rank-sum and between 3 or more groups by Kruskal-Wallis with post hoc testing by Dunn's pairwise comparison for variables significantly different by Kruskal-Wallis.

*Significant difference between groups by Kruskal-Wallis test ($p < 0.05$)

$^{\alpha}$ Significant difference between CKRT intervention and CKRT controls by post hoc test, Dunn's pairwise comparison ($p < 0.05$)

$^{\beta}$ Significant difference between CKRT controls and ICU controls by post hoc test, Dunn's pairwise comparison ($p < 0.05$)

$^{\gamma}$Significant difference between baseline and follow-up by Wilcoxon rank-sum ($p < 0.05$)

ARDS acute respiratory distress syndrome, *BMT* bone marrow transplant, *DIC* disseminated intravascular coagulation, *FIRES* febrile infection-related epilepsy syndrome, *HUS* hemolytic uremic syndrome, *SBO* small bowel obstruction, *SIRS* systemic inflammatory response syndrome

baseline to − 27.6% at follow-up, which was similar to that of healthy controls (− 25.7%). Meanwhile the circumferential strain of the CKRT Control group, that did not receive carnitine supplementation, remained impaired (− 20.3%) at follow-up, in comparison to both the carnitine supplemented group and the healthy controls.

Discussion

This is the first study to demonstrate that IV carnitine supplementation is associated with improvement in myocardial strain and repletion of plasma total and free carnitine in children with AKI receiving CKRT. At time of initiation of CKRT, all patients exhibited impairment in longitudinal and circumferential myocardial strain, as compared to healthy controls. After ≥ 1 week, CKRT patients who received IV carnitine supplementation demonstrated improvement in longitudinal and circumferential myocardial strain, while strain of the patients on CKRT who did not receive carnitine supplementation remained impaired. Furthermore, the CKRT patients not on carnitine supplementation exhibited marked total and free plasma carnitine deficiency after ≥ 1 week, while the carnitine supplemented CKRT patients were repleted. In

comparison, the children in the ICU Control group (no AKI, no CKRT) were able to maintain total and free plasma carnitine levels within normal range, despite having no source of exogenous carnitine for ≥ 1 week. Our findings suggest that pediatric patients receiving CKRT are at high risk for carnitine deficiency and may benefit from supplementation.

In healthy individuals, carnitine is obtained primarily through dietary intake of dairy and meat products and is stored in the cardiac and skeletal muscle, where it plays a key role in energy production. Although little was previously known about carnitine status in patients with AKI receiving CKRT, iatrogenic carnitine deficiency related to chronic hemodialysis in patients with CKD 5 is a well-known phenomenon. As plasma-free carnitine is low in molecular weight and non-protein bound, its level decreases by approximately 70% with each hemodialysis treatment [2]. Re-equilibration of plasma carnitine from muscle stores occurs post-dialysis, but when plasma carnitine losses are unable to be matched by synthesis and movement out of tissues, depletion of skeletal muscle carnitine stores occurs [22]. Therefore, chronic hemodialysis results in progressive decline in plasma and muscle carnitine over time and [22] carnitine deficiency has been documented in the pediatric chronic hemodialysis population [17, 23–27].

However, surprisingly little is known about carnitine deficiency, supplementation, and outcomes in patients with AKI receiving CKRT. We hypothesized that given the continuous removal of solutes by CKRT in combination with lack of dietary intake and impaired production of endogenous carnitine by the kidney in critically ill children with AKI, carnitine would be rapidly depleted. Two small studies on the kinetics of carnitine removal in patients receiving CKRT confirmed complete passage and efficient removal of free carnitine through the CKRT dialysis membrane, based on sieving coefficients [28, 29]. As reported by Chadha et al., in patients receiving TPN and CKRT, the sieving coefficient of carnitine is > 84% and carnitine losses approximately 80% of carnitine intake [29]. Given the rapid clearance of free carnitine by CKRT, combined with the lack of intake (as carnitine is not included in standard TPN solutions), it stands to reason that the potential for carnitine deficiency and need for supplementation in this population would be profound.

To this point, we previously demonstrated rapid progression of carnitine deficiency in 42 pediatric patients receiving CKRT in a prior study [18]. In that study, approximately two-thirds of the CKRT patients were found to be total and free carnitine deficient after 1 week on CKRT, 80% were deficient

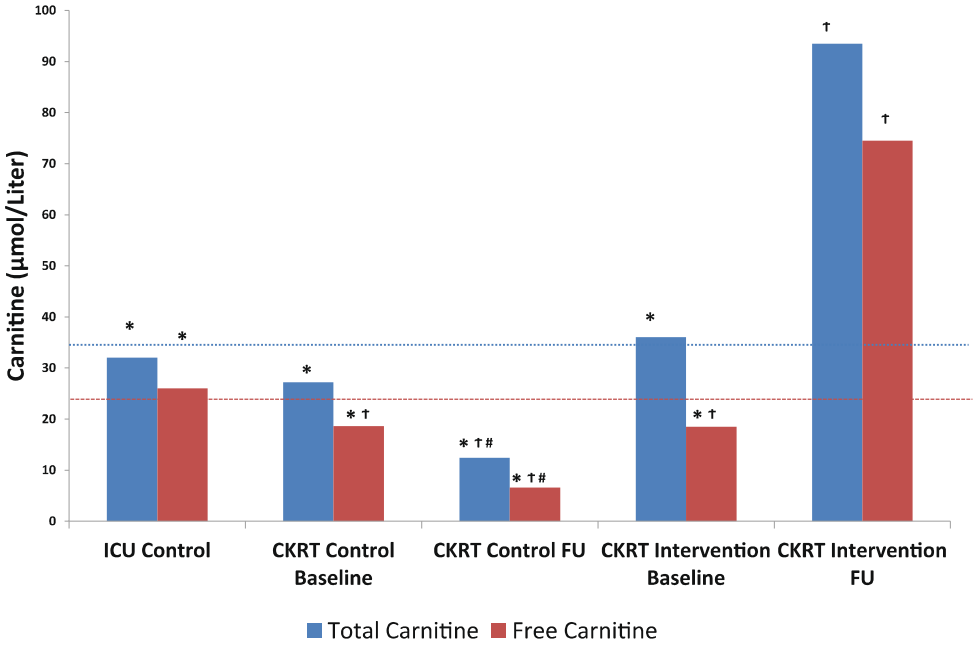


Fig. 2 Plasma total and free carnitine levels

Median levels of plasma total carnitine (blue bars) and free carnitine (red bars) are shown. Horizontal dashed lines indicate the most conservative normal cut points for total carnitine (blue line) and free carnitine (red line). Carnitine levels were compared between and within groups by Wilcoxon rank-sum, with $p < 0.05$ considered significant.

*Indicates significant difference in carnitine levels as compared to CKRT carnitine-supplemented group at follow-up.

†Indicates significant difference in carnitine levels as compared to ICU control group.

#Indicates significant difference in carnitine levels as compared to CKRT control group at baseline.

Continuous kidney replacement therapy (CKRT), Intensive care unit (ICU), Follow-up (FU)

Table 2 Comparison of longitudinal strain of children in CKRT carnitine intervention and CKRT Control groups and healthy controls

Longitudinal myocardial strain[a]	CKRT intervention median (IQR)	CKRT control median (IQR)	Healthy controls median (IQR)	*p* value CKRT intervention vs. CKRT control	*p* value CKRT intervention vs. healthy control	*p* value CKRT control vs. healthy control
Baseline (pre-CKRT initiation)	− 18.5 (− 19.4, − 16.2)	− 13.8 (− 19.1, − 11.8)	− 22.6[c] (− 25.3, − 20.9)	0.21	0.0003*	0.0006*
Follow-up[b] (on CKRT ≥ 1 week)	− 21.1 (− 27.7, − 20.7)	− 14.4 (− 20.2, − 12.7)		0.004*	0.87	0.002*
p value within groups (baseline vs. follow-up)	0.005*	0.93				

[a] More negative strain values signify better myocardial contractility

[b] Difference in duration of CKRT at time of strain measurement was not statistically significant as compared between the intervention and control groups (11 [IQR 10,14] vs. 13 [IQR 8,18] days $p = 0.12$)

[c] Healthy controls had myocardial strain measured at one time point, which was compared to both baseline and follow-up strain of the CKRT groups

*Significant difference in myocardial strain by Wilcoxon rank-sum

Table 3 Comparison of circumferential strain of children in CKRT intervention and CKRT Control groups with and healthy controls

Circumferential myocardial strain[a]	CKRT intervention median (IQR)	CKRT control median (IQR)	Healthy controls median (IQR)	*p* value CKRT intervention vs. CKRT control	*p* value CKRT intervention vs. healthy control	*p* value CKRT control vs. healthy control
Baseline (pre-CKRT initiation)	− 18.3 (− 28.1, − 15.0)	− 19.0 (− 26.2, − 13.7)	− 25.7 (− 28.6, − 24.6)	0.84	0.05*	0.05*
Follow-up[b] (on CKRT ≥ 1 week)	− 27.6 (− 31, − 26)	− 20.3 (− 14.9, − 24.9)		0.02*	0.09	0.008*
p value within groups (baseline vs. follow-up)	0.03*	0.95				

[a] More negative strain values signify better myocardial contractility

[b] Difference in duration of CKRT at time of strain measurement was not statistically significant as compared between the intervention and control groups, (11 [IQR 10,14] vs. 13 [IQR 8,18] days $p = 0.12$)

[c] Healthy controls had myocardial strain measured at one time point, which was compared to both baseline and follow-up strain of the CKRT groups

*Significant difference in myocardial strain defined by Wilcoxon rank-sum

at 2 weeks, and 100% of patients were carnitine deficient beyond 3 weeks of initiation of CKRT [18]. In the present study, CKRT patients who did not receive carnitine supplementation were indeed carnitine deficient after ≥ 1 week on CKRT and had significantly lower plasma total and free carnitine levels as compared to the CKRT patients who received daily carnitine supplementation added to TPN.

There is some evidence in the literature to suggest that carnitine deficiency can occur in the setting of critical illness and sepsis alone [30, 31]. In order to account for this variable, our study included an ICU Control group of critically ill TPN-dependent children who did not have advanced kidney failure and did not receive CKRT. Despite critical illness and TPN-dependence without any exogenous carnitine intake for ≥ 1 week, the ICU control group maintained total and free carnitine levels in normal range, and their levels were significantly higher as compared to the unsupplemented CKRT group. This illustrates that the risk of iatrogenic CKRT-related carnitine deficiency is more severe compared with that related to isolated factors such as critical illness or lack of dietary intake.

Given the rapid depletion of carnitine by CKRT and the important role of carnitine in providing energy to the myocardium, we studied the impact of carnitine deficiency on myocardial function. In our study, CKRT patients who received carnitine supplementation demonstrated better myocardial contractility, as defined by better strain, compared with those who did not receive carnitine. This finding can be explained by the fact that carnitine plays a key role in supplying energy to the myocardium. In the setting of carnitine deficiency, energy production is impaired, negatively impacting cardiac contractility. Repletion of carnitine stores via supplementation in TPN may in turn promote increased energy production in cardiac muscle, thereby improving myocardial contractility in children receiving CKRT [5]. Proof of this concept has been demonstrated in both animal and human studies. In animals, carnitine therapy prevented cardiomyocyte apoptosis [32] and increased adenosine triphosphate (ATP) production in ischemic dog hearts [33]. In adult chronic HD patients, numerous studies reported improved LV function, evidenced by increased ejection fraction, in response to carnitine supplementation [4–7]. In children, cardiac changes may be more subtle, as we previously demonstrated improvement in myocardial strain in response to IV carnitine supplementation in children on chronic HD who had normal ejection fraction [17]. In that study, the improvement in longitudinal strain rate was detected by speckle tracking echocardiography, but was not evident by standard echocardiography parameters, such as ejection fraction.

A major strength of our study was the use of speckle tracking echocardiography to assess myocardial function. Speckle tracking echocardiography is a highly sensitive technology that detects regional myocardial motion by tracking natural acoustic markers (speckles) within the myocardium in the longitudinal and circumferential directions. Speckle tracking echocardiography is superior to standard echocardiography due to its ability to detect early signs of regional myocardial dysfunction [34]. In addition, because speckle tracking echocardiography measures myocardial deformation directly, it is significantly less affected by volume changes or ventricular loading conditions; therefore, speckle tracking echocardiography is particularly well suited for patients with conditions such as kidney dysfunction and sepsis [35, 36]. This is an advantage over standard echocardiographic measures such as ejection fraction, which are highly impacted by ventricular preload and afterload conditions. Impaired strain has been associated with increased risk of mortality [37, 38], while improved strain is predictive of recovery following myocardial infarction in adults [39].

Our pilot study was single center and limited by small sample size. The small sample size may have limited our ability to detect significant differences in demographics and clinical characteristics, and we were not able to perform multivariable analyses. However, given that it is a pilot study, our findings provide a solid launching point for future investigations. Although we were not able to adjust for confounding variables statistically, our study design was strengthened by the comparison of the intervention group with three different control groups, which helps to tease out whether changes in plasma carnitine status and myocardial strain may be related to carnitine supplementation, CKRT, or to critical illness alone. Performance of speckle tracking echocardiography in healthy controls was important to provide age-matched reference data, since normal reference ranges for myocardial strain in children have not been established. Collection of plasma carnitine levels of ICU control patients provided a reference for carnitine status in children who are critically ill without the exposure of CKRT. It would have been interesting to also perform echocardiograms in the ICU controls; however, this was not feasible due to budget constraints. Despite inclusion of the three control groups, we acknowledge that we were likely not able to control for all possible factors that could influence myocardial strain in a critically ill pediatric population. Larger randomized controlled clinical trials are needed in order to more definitively prove a causal relationship between carnitine supplementation and improved myocardial function in children receiving CKRT.

In conclusion, children receiving CKRT are at high risk for carnitine deficiency. Supplementation with IV carnitine is associated with repletion of plasma carnitine and improvement in myocardial strain. While larger studies are needed to further investigate clinical outcomes associated with carnitine supplementation, the findings of the present study suggest that IV carnitine supplementation may benefit pediatric patients undergoing a prolonged course of CKRT.

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Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request, upon approval of a Data Use Agreement, as per institutional regulations.

Compliance with ethical standards

Conflict of interest The PI of the study, Asha Moudgil, MD, received a research grant from Leadiant Biosciences Inc., as noted above.

Ethics approval All procedures were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by the Institutional Review Board at Children's National Hospital (protocol number 3555).

Consent to participate Informed consent was obtained from the parents of the participants in the CKRT Intervention group, the ICU Control group, and the Healthy Control Group.

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