

# Plasma Fatty Acid Levels in Children during Extracorporeal Membrane Oxygenation Support—A Pilot Study

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**Abstract:** Plasma fatty acids levels are markedly elevated in patients with myocardial ischemic–reperfusion injury including those after cardiopulmonary bypass (CPB). High levels of fatty acids have detrimental effects on myocardial function. Extracorporeal membrane oxygenation (ECMO) is like CPB, but much longer, to provide a life-saving support for patients with cardiac arrest. We measured plasma fatty acid levels in children during ECMO support. Five children (aged .3–36 months, median 20 months) receiving venoarterial ECMO support after cardiac arrest in 2010 and 2011 were enrolled. The study was initiated at 32–56 hours after the start of ECMO support as a result of the complicated clinical scenario. Fatty acids were measured at 8-hour intervals for 1–3 days. The dosage of inotropes and vasoactive agents was recorded concurrently. The duration of ECMO ranged from 70 to 240 hours (median 177 hours). Four patients were successfully weaned off ECMO support. One died after termina-

tion of ECMO. Levels of fatty acids were elevated compared with the normal values. Overall, fatty acid levels continuously decreased over time ( $p < .0001$ ), the mean being  $1.03 \pm .33$  mmol/L in 30–50 hours,  $1.01 \pm .57$  in 50–70 hours,  $.81 \pm .32$  in 70–90 hours, and  $.63 \pm .23$  hours. No correlation was found between fatty acid levels and other clinical variables, including age, dosage of inotropes and vasoactive agents, or ECMO duration. Plasma fatty acids levels are elevated in children during ECMO support and continuously decrease over time. Fatty acid levels may be markedly higher in the immediate hours after the initiation of ECMO. Data from more patients are needed to understand the profiles of fatty acids and the correlations with clinical variables. Metabolic manipulations to decrease fatty acids might improve myocardial recovery in patients undergoing ECMO support. **Keywords:** extracorporeal membrane oxygenation, plasma fatty acids, pediatric. *JECT. 2013;45:242–247*

Plasma fatty acid levels are elevated and often sustained in patients with ischemia–reperfusion injury and subsequent systemic inflammatory and metabolic response (1–6). This phenomenon has been observed in adult patients after acute myocardial infarction (7) and adults and children after cardiopulmonary bypass surgery (CPB) (8,9). High levels of fatty acids have been shown to be harmful both experimentally (10,11) and clinically (8,12), contributing to myocardial injury, functional

impairment, and mortality (13–15). The detrimental effects of fatty acids result from the inhibition of myocardial glucose oxidation (16,17) or to the accumulation of toxic intermediates of fatty acid metabolism (18). Our group has found that the levels of fatty acids in children undergoing CPB remain markedly elevated by 24 hours postoperatively (8). Extracorporeal membrane oxygenation (ECMO) is commonly used to provide a temporary life-saving support for patients with cardiac arrest. CPB and ECMO share a common interaction of blood with the artificial surfaces of the system and thus a common mechanism for inducing systemic inflammatory and metabolic response (19,20). Different from CPB, the duration of ECMO support is much longer, for days or weeks, instead of hours of CPB. Therefore, ECMO may induce a persistent systemic inflammatory and metabolic response. However, the changes of fatty acids in children during ECMO support remain unknown.

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In the current study, we aimed to obtain information regarding the levels of plasma fatty acids in children during ECMO support.

## PATIENTS AND METHODS

### Patients

The study was approved by the Institutional Health Research Ethics Board at the Stollery Children's Hospital, Edmonton, Alberta, Canada. Written informed consents were obtained from the parents of five children (three girls and two boys, age .3–36 months, median 20 months) receiving ECMO support after cardiac arrest between 2010 and 2011. Demographic data are shown in Table 1. All patients received venoarterial ECMO.

### Critical Care

Patients received time-cycled pressure control/pressure support ventilation during the ECMO support. Central body temperature was maintained at 36–37°C. ECMO flow was adjusted and inotropes and vasoactive agents were given to maintain the systolic blood pressure higher than 65 mmHg. Inotropes and vasoactive agents included epinephrine (.005–.5 µg/kg/min, n = 4), norepinephrine (.05 µg/kg/min, n = 1), milrinone (.05–.75 µg/kg/min, n = 4), and nitroprusside (2.0–5.0 µg/kg/min, n = 1). The blood gas management protocol was to maintain PaO<sub>2</sub> at 100–200 mmHg, PaCO<sub>2</sub> at 40–50 mmHg, pH at 7.35–7.45, and SvO<sub>2</sub> at 70%. Hemoglobin was maintained at 120 mg/L by giving 5–10 mL/kg red blood cells or 5–10 mL/kg plasmalyte. The glucose management protocol was to administer insulin when blood glucose exceeded 15 mmol/L. Arterial glucose concentrations and the dosage of inotro-

pic and vasoactive agents were recorded concurrently with fatty acid measurements. Heparin was given at the rate of 10–22 units/kg/h in all patients.

### Measurement of Plasma Fatty Acids

Blood samples for the measurement of fatty acids were drawn at 8-hour intervals in the 72-hour study period. After spinning at 900 rpm for 15 minutes at 4°C, blood samples were stored at –80°C. The concentrations of fatty acids were measured using the Roche Free fatty acid, Half-micro test kit. The assay is a coupled enzymatic reaction using acyl coenzyme A synthetase and acyl CoA oxidase to convert free fatty acids into enoyl coenzyme A and hydrogen peroxide. The hydrogen peroxide in turn oxidizes 4-aminoantipyrine forming a red dye that absorbs at 546 nm.

### Measurement of Arterial Glucose Concentration

Arterial glucose concentration was measured using a blood gas analyzer (ABL 700; Radiometer Copenhagen, Copenhagen, Denmark).

### Study Protocol

As a result of the varied time of consenting and cardiac functional recovery in the complicated clinical scenario in this particular group of patients, in terms of child's critical illness, emergent nature, and parents' distress and their time to resolve and give the consent, the time of initiation and termination of the study varied among patients. The study was initiated at the 30th to 56th hour (median 49th) on ECMO. The protocol was to continue the study for consecutive 72 hours. Measurements of fatty acids and other clinical variables were collected at 8-hour intervals. Four patients completed the entire study period. Patient 1 was studied for 12 hours.

**Table 1.** Demographic and clinical data.

Patient No.	1	2	3	4	5	Mean ± SD
Gender	M	F	F	M	F	
Age (month)	3	36	20	.3	22	16.3 ± 14.7
Weight (kg)	6	16	13	2.6	11	9.7 ± 5.4
Diagnosis	Multifocal atrial tachycardia; cardiac arrest	Acute lymphoblastic leukemia; cardiac arrest	Myocarditis; cardiac arrest	Right atrial isomerism, bilateral superior vena cava; postcardiac surgery, cardiac arrest	RV-dominant AVSD, hypoplastic aortic arch; post cardiac surgery, cardiac arrest	
ECMO type	V-A	V-A	V-A	V-A	V-A	
ECMO duration (hours)	70	177	120	216	240	165 ± 70
ICU stay (days)	9	22	7	33	35	21 ± 13
Hospital stay (days)	13	22	8	33	49	25 ± 16
Deceased	No	No	No	Yes	No	

SD, standard deviation; ECMO, extracorporeal membrane oxygenation; ICU, intensive care unit; M, male; F, female; RV, right ventricle; AVSD, atrioventricular septal defect; V-A, venoarterial.

**Data Analysis**

Data are expressed as mean ± standard deviation or range and median. Mixed linear regression for repeated measures was used to determine the nature of any time trend of the variables during the study period. Mixed linear regression for repeated measures was also used to analyze correlations of fatty acid levels with other clinical variables. All data analysis was performed with SAS statistical software Version 9.2 (SAS Institute, Inc., Cary, NC). *p* < .05 was considered significant.

**RESULTS**

The duration of ECMO support was 160 ± 70 hours (range 70–240 hours, median 177 hours). Four patients completed the study, except Patient 1 who was studied for 12 hours and then was weaned from ECMO. Four patients were successfully weaned from ECMO support. One patient (Patient 4) was weaned off ECMO after 216 hours of support but died on the 27th day after ECMO termination as a result of intracranial hemorrhage. None of the patients received insulin during the study period.

The values of fatty acid level in each patient at each time of measurements, together with clinical measurements, are shown in Table 2. The first measurement of fatty acids was obtained from the 30th to 56th hour (median 49th) since the initiation of ECMO. Overall, the level of fatty acids continuously decreased over the next 72-hour study period (*p* < .0001), the mean being 1.03 ± .33 mmol/L at 30–50 hours, 1.01 ± .57 at 50–70 hours, .81 ± .32 at 70–90 hours, and .63 ± .23 at 90–120 hours. Arterial glucose concentration did not change significantly (*p* = .15), the mean being 7.8 ± 2.3 mmol/L at 30–50 hours, 7.8 ± 2.5 mmol/L at 50–70 hours, 7.5 ± 1.4 mmol/L at 70–90 hours, and 7.4 ± 1.9 mmol/L at 90–120 hours. No correlation was found between fatty acid levels and other clinical variables, including age, arterial glucose concentration, dosage of inotropic and vasoactive agents, or ECMO duration.

**DISCUSSION**

This study provides the first information about the changes of plasma fatty acid levels in children during ECMO support. Our data demonstrate that plasma fatty acids are elevated with a gradual decrease over the time during ECMO support.

Fatty acid levels are elevated and often sustained in all of the common clinical situations of ischemia–reperfusion and subsequent systemic inflammatory and metabolic response. The metabolic response is characterized by increased catabolism as a result of increased endogenous catecholamine release and decreased insulin release with increased insulin resistance, leading to lipolysis, thus

**Table 2.** Plasma fatty acid levels and clinical variables during the 72-hour study period in five children on ECMO.

	Patient No.					
	1	2	3	4	5	
First 24 hours	Hours on ECMO	30	38	56	64	72
	FFA (mmol/L)	1.434	1.526	1.142	1.513	.779
	Temperature (°C)	36.7	36.8	36.9	36.8	36.8
	mBP (mmHg)	67	68	61	56	51
24–48 hours	pH	7.42	7.44	7.48	7.42	7.43
	ECMO flow (L/min)	1.1	.81	1.18	1.18	1.11
	Hours on ECMO	50	58	80	88	96
	FFA (mmol/L)	1.072	1.274	.912	.797	.168
48–72 hours	Temperature (°C)	36.7	36.7	36.9	36.9	36.9
	mBP (mmHg)	67	64	74	82	57
	pH	7.47	7.45	7.52	7.39	7.47
	ECMO flow (L/min)	.55	.6	.51	1.08	1.03
	Hours on ECMO	72	80	104	112	120
	FFA (mmol/L)	1.415	1.103	.735	.668	.518
	Temperature (°C)	36.8	36.8	36.5	37.1	37.1
	mBP (mmHg)	71	71	81	64	64
	pH	7.53	7.52	7.52	7.44	7.37
	ECMO flow (L/min)	.33	.74	.67	.57	.14

ECMO, extracorporeal membrane oxygenation; FFA, free fatty acids; mBP, mean arterial blood pressure.

elevated levels of plasma fatty acids (21,22). Reperfusion of severely ischemic myocardium exposes the myocardium to high levels of fatty acids during a critical period of clinical recovery (7,23). Experimental studies from our group and others have shown that high levels of fatty acids are detrimental to the mechanical functional recovery of previously ischemic hearts (16,18) as a result of the inhibition of myocardial glucose oxidation (16,17) and the accumulation of toxic intermediates of fatty acid metabolism (18). In adult patients with acute myocardial infarction, high levels of fatty acids are thought to contribute to infarct size, arrhythmia, and mortality (13–15).

Our group has further studied the changes of fatty acids in infants and children after CPB. In the immediate hours after surgery, fatty acids levels increased from  $.81 \pm .10$  mmol/L as seen in the control group of infants to  $3.27 \pm .26$  mmol/L during aortic cross-clamping and remained elevated during immediate reperfusion ( $1.91 \pm .15$  mmol/L) and for 24 hours after surgery ( $1.67 \pm .22$  mmol/L) (8). A number of factors may account for the high levels of fatty acids in patients undergoing CPB: 1) heparin administration during CPB, resulting in a release of fatty acids from lipoproteins as a result of increased lipoprotein lipase activity; 2) systemic inflammatory and metabolic response subsequent to interaction of blood with the artificial surfaces of the system and ischemia–reperfusion, leading to hormonal alterations with increased endogenous catecholamine release and decreased insulin release with increased insulin resistance (1–6); 3) exogenous catecholamine administration, resulting in release of fatty acids into the circulation from adipose tissue triglyceride lipolysis; and 4) the volume or metabolic status of the patient. Additionally, free fatty acid levels in infants and children dramatically increased at the onset of anesthesia to approximately 1.8 mmol/L, which is likely related to the stress they experienced before surgery (8). Lastly, it should be noted that in our previous study, the control group of six infants was admitted to the neonatal intensive care unit but not requiring open heart surgery. Fatty acid levels in these infants were twice those seen in normal adult subjects, perhaps simply because these infants experienced problems that may contribute to increased plasma fatty acid levels. The normal level of free fatty acids may be  $.48 \pm .01$  mmol/L (24).

ECMO is commonly used to provide temporary life-saving support for patients with cardiac arrest. ECMO and CPB share almost of all the common factors mentioned to induce elevated levels of plasma fatty acids. Differently, the duration of ECMO is much longer, for days (e.g., 3–10 days in our patients) instead of hours of CPB. Our data showed that mean level of fatty acids was  $1.03 \pm .33$  mmol/L in 30–50 hours and remained at this level in the next 20 hours, suggesting persistently elevated levels of fatty acids during ECMO as compared with normal children (8). Fatty acid levels significantly decreased

over time to the normal range thereafter in 70–120 hours on ECMO. The peak level of fatty acids in the patients on ECMO in the current study is considerably lower than that in our previous study in children in the postoperative period after CPB (8). This may be largely the result of the delayed time of the first sample collection, that is, 30–56 hours after the initiation of ECMO rather than within 24 hours after CPB in a previous study. The actual levels of fatty acids in the immediate hours on ECMO may be expected to be markedly higher. During the same period, blood glucose concentration was persistently higher than the normal range in our patients indicating decreased insulin release or with increased insulin resistance. Additionally, our data did not show any statistical correlation between the fatty acid levels and other clinical variables including blood glucose concentration, the dosage of inotropic and vasoactive agents, or the duration of ECMO support. This may be the result of the small number of patients.

The report of our pilot data indicates the end of beginning of the investigation of fatty acids in patients on ECMO. Further studies are necessary to obtain a better understanding of the changes of fatty acid levels, the affecting factors in the current clinical management, and the correlation with myocardial functional recovery. This is important because systemic inflammatory and metabolic response play a central role in determining clinical outcomes (21,22,25–28) and has been shown to be associated with adverse effects in neonates with respiratory failure treated with ECMO (29). Treatment strategies to manipulate metabolism may potentially improve myocardial function in this particular group of patients who have cardiac arrest. For example, pharmacologic stimulation of glucose oxidation during reperfusion may overcome the detrimental effects of fatty acids on myocardial functional recovery (17,30,31).

## LIMITATIONS

Our study was carried out in a small number of patients, and the measurements of plasma fatty acids were started 30–56 hours after the initiation of ECMO as a result of the complicated clinical situations in this particular group of critically ill patients. Our data showed a trend of elevated fatty acids and the gradual decrease over time rather than a complete picture of the profile of fatty acids during the entire period of ECMO. We did not find any correlations between fatty acids and clinical variables such as blood glucose concentration, or the dosage of inotropes, likely as a result of the small number of patients. In addition, the measurement of fatty acids was performed using an enzymatic and colorimetric method. This method only provides the total concentration of fatty acids but not the relative amounts of saturated and unsaturated fatty acids. It has been demonstrated that varied types of fatty acids may



have different effects on metabolism (32,33). In addition, saturated and unsaturated fatty acids seem to have specific and differential effects on muscle insulin signaling in vitro (34–36), evident even in biopsies derived from children (37). Thus, measurements of different fatty acids types may provide specific information for the development of potential treatment strategies to improve myocardial function and clinical outcomes.

## CONCLUSIONS

Plasma fatty acids levels are elevated in children during ECMO support postcardiac arrest and continuously decrease over time. Fatty acid levels may be markedly higher in the immediate hours after the initiation of ECMO. Data from more patients are needed to understand the profiles of fatty acids and the correlations with clinical variables. Metabolic manipulations to decrease fatty acids might improve myocardial recovery in patients undergoing ECMO support.

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