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Original article

Extracorporeal membrane oxygenation promotes long chain fatty acid oxidation in the immature swine heart in vivo

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ABSTRACT

Extracorporeal membrane oxygenation (ECMO) supports infants and children with severe cardiopulmonary compromise. Nutritional support for these children includes provision of medium- and long-chain fatty acids (FAs). However, ECMO induces a stress response, which could limit the capacity for FA oxidation. Metabolic impairment could induce new or exacerbate existing myocardial dysfunction. Using a clinically relevant piglet model, we tested the hypothesis that ECMO maintains the myocardial capacity for FA oxidation and preserves myocardial energy state. Provision of 13-Carbon labeled medium-chain FA (octanoate), long-chain free FAs (LCFAs), and lactate into systemic circulation showed that ECMO promoted relative increases in myocardial LCFA oxidation while inhibiting lactate oxidation. Loading of these labeled substrates at high dose into the left coronary artery demonstrated metabolic flexibility as the heart preferentially oxidized octanoate. ECMO preserved this octanoate metabolic response, but also promoted LCFA oxidation and inhibited lactate utilization. Rapid upregulation of pyruvate dehydrogenase kinase-4 (PDK4) protein appeared to participate in this metabolic shift during ECMO. ECMO also increased relative flux from lactate to alanine further supporting the role for pyruvate dehydrogenase inhibition by PDK4. High dose substrate loading during ECMO also elevated the myocardial energy state indexed by phosphocreatine to ATP ratio. ECMO promotes LCFA oxidation in immature hearts, while maintaining myocardial energy state. These data support the appropriateness of FA provision during ECMO support for the immature heart.

1. Introduction

Extracorporeal membrane oxygenation (ECMO) provides a form of rescue for infants and children with severe pulmonary or cardiac disease [1,2]. Respiratory failure and/or pulmonary hypertension which occur with diaphragmatic hernia are the primary indications in neonates, although ECMO is used predominantly for severe acute cardiac decompensation in older infants and children [2]. Veno-arterial (V-A) ECMO redirects systemic venous return into a mechanical circuit containing an oxygenator. A pump within the circuit returns oxygenated blood to a major artery and maintains mean systemic blood flow pressure, while markedly reducing aortic pulse pressure. Thus, V-A ECMO provides a form of biventricular pressure and volume unloading, which theoretically allows the heart to rest and recover from injury. However, ECMO can induce a cardiac stun syndrome starting within a few hours of instituting support [3–6]. Stunning evidenced by severe cardiac dysfunction occurs in infants and children even without prior cardiac injury [4,5], and similarly occurs in ECMO animal models [7]. Occurrence of stun impedes weaning or removal from the circuit which is the overall goal of therapy.

The mechanisms responsible for stunning still require elucidation. Some investigators have suggested that a surge in proinflammatory cytokines induced by the circuit is at least partially responsible [8–10]. In particular, we have previously noted that ECMO performed in immature swine promotes a tremendous increase in plasma interleukin-6 levels [11]. This particular proinflammatory cytokine decreases insulin sensitivity, possibly explaining skeletal muscle wasting noted during infant ECMO despite relatively high caloric supply [12]. However, the impact of ECMO on cardiac substrate metabolism has not been previously elucidated. The heart for many species undergoes an early postnatal metabolic shift towards preference for fatty acid (FA) oxidation [13,14]. This shift is followed by further marked inhibition of carbohydrate oxidation during the period of rapid cardiac growth. Accordingly, the immature heart depends primarily on fats for provision of oxidative substrate to the citric acid cycle (CAC). Both medium- and long-chain FAs within emulsions are typically infused intravenously as fuel for infants and children undergoing ECMO procedures [15,16]. However, it is unclear if the heart can sustain a further shift towards FA oxidation under ECMO conditions which

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may inhibit glucose oxidation. ECMO impairment of cardiac substrate oxidation could lead to reductions in mitochondrial ATP production or to a lower myocardial energy state, which could both explain the contractile dysfunction associated with the stun phenomenon. We tested the hypothesis that the ECMO support for the immature heart maintains the myocardial capacity for FA oxidation and thereby preserves the myocardial energy state. Using an immature piglet model emulating V-A ECMO in infants and children, we first determined if ECMO induced shifts in utilization of the three principal substrates oxidized by the pig myocardium: lactate, medium-chain FA (MCFA), exemplified by octanoate, and long-chain mixed FAs (LCFAs). Then we determined if the heart was capable of increasing relative acetyl-CoA contribution to the CAC from these substrates after direct perfusion infusion into the coronary arteries. Nuclear magnetic resonance (NMR) methods were used to determine substrate fractional contributions and metabolomic profiles in these hearts. Myocardial energy state was indexed by phosphocreatine (PCr) to ATP ratio, a typical surrogate for phosphorylation potential.

2. Methods

2.1. Animals

All experimental procedures were approved by Seattle Children's Institutional Animal Use and Care Committee. Twenty-two male Yorkshire piglets (body weight 10.6–15.6 kg, age 25–38 days) were used. They were fasted overnight with free access to water. They were premedicated with an intramuscular injection of ketamine (33 mg/kg) and xylazine (2 mg/kg). After intubation through surgical tracheostomy, the piglets were mechanically ventilated (FiO_2 40–60%, volume control 15 mL/kg, PEEP 3cmH_2O, respiratory rate 14–18/min), and isoflurane (1–2%) to maintain general anesthesia. An arterial pCO_2 of 35–45 mm Hg was maintained by adjusting minute ventilation.

2.2. Protocol

Animals were separated into experimental groups first by mode of circulation either normal without extracorporeal support (CON) or ECMO which provides mechanical unloading. The ECMO circuit is described below. Sham animals (CON) received anesthesia, assisted ventilation, and heparinization similar to ECMO animals, but were not connected to the ECMO circuit. Within each of these groups the animals were further separated by mode of 13-Carbon (^{13}C) substrate delivery either in tracer amounts through systemic venous route (CON-S; ECMO-S) or delivery at high concentration to the heart via intracoronary (CON-IC; ECMO-IC) infusion into the left anterior descending coronary artery (LAD). Animals were maintained for 8 h and received steady-state ^{13}C substrate infusions over the final hour. Immediately upon completion of the labeled infusion, the portions of left ventricular (LV) myocardium perfused by the LAD were rapidly freeze-clamped and stored under liquid nitrogen for later extraction. Arterial and coronary venous blood samples were collected at multiple time points: after anesthesia induction and before ECMO as a baseline, 1, 2, 4 and 7 h after starting ECMO, and just before completion of the labeled infusion as an endpoint. Baseline data was obtained after administration of heparin. Blood samples were immediately centrifuged and aliquots of plasma were stored at −80 °C. Plasma lactate and free FA concentrations were measured using commercial kits (BioVision, Mountain View, CA and Cayman, Ann Arbor, MI). Blood glucose was measured using a Bayer Contour point-of-care glucometer (Bayer HealthCare, Tarrytown, NY). Blood pH, pCO_2, pO_2, and hemoglobin were measured at regular intervals by a Radiometer ABL 800 (Radiometer America, Westlake, OH). Myocardial oxygen consumption (MVO_2) was calculated from coronary venous flow and blood gas analysis. Ventilator settings as described above were maintained during ECMO.

2.3. Hemodynamic monitoring

An arterial line for systemic blood pressure monitoring and blood sampling was placed in the femoral artery. A saline-filled catheter was inserted into the internal jugular vein for continuous heparin infusion and connected to a pressure–membrane transducer for recording of the central venous pressure. A flow probe was placed around the ascending aorta to measure cardiac output (TS420, Transonic Systems Inc., Ithaca, NY). A 5-French high-fidelity micromanometer (Millar Instruments, Houston, TX) was inserted via the apex to measure LV pressure. To measure coronary venous flow, a cannula with an inflatable balloon cuff was placed into the coronary sinus via the right atrium; blood was returned to the superior vena cava by a shunt loop. A Transonic flow probe was placed around this shunt for continuous flow monitoring. The hemiazygos vein, which drains systemic venous blood to the coronary sinus in swine, was ligated to avoid systemic contamination of the coronary venous blood. A PowerLab 16/30 recorder (AD Instruments Inc., Colorado Springs, CO) continuously recorded hemodynamic data in all cases.

2.4. ECMO circuit and management

We used a miniaturized extracorporeal circuit to minimize hemodilution and avoid the need for blood transfusions. The circuit consisted of the following: a roller peristaltic pump console (Sarn8000 Terumo, Tokyo, Japan); a hollow fiber membrane oxygenator (CX-RX05RW, Terumo, Tokyo, Japan). The circuit was primed with dextran 40 in 0.9% sodium chloride, 5% dextrose and 2000 units of heparin. The total prime volume was 80 mL.

After median sternotomy, the ascending aorta and right atrium were cannulated to create a V-A ECMO circuit. Management during ECMO maintained the pump flow rates of 80–100 mL/kg/min. We maintained a pH of 7.35 to 7.45, an arterial pCO_2 of 35 to 45 mm Hg, pO_2 of >100 mm Hg and a rectal temperature of 36 to 37.5 °C. ECMO duration time was 8 h.

2.5. Infusion of labeled substrates

As noted previously, two separate substrate delivery methods were used. [2-^{13}C]lactate and [2,4,6,8-^{13}C]octanoate, MCFA, were obtained from Sigma (St. Louis, MO), and [U–^{13}C]LCFAs were obtained from Cambridge Isotope Laboratories (Andover, MA). LCFAs consist of palmitic acid (45–55%), palmitoleic acid (10–15%), oleic acid (20–30%) and linoleic acid (10–15%). Labeled substrates in all protocols were infused for the final 60 min of the protocol. For the systemic delivered dose, [2-^{13}C]lactate [2,4,6,8-^{13}C]octanoate and [U–^{13}C]LCFAs were used at 2.6, 0.8 and 0.8 μmol/kg body weight/min respectively, and were delivered into the left atrium (CON-S) or the aortic return cannula (ECMO-S). Intracoronary final concentrations of metabolites are equal to systemic arterial plasma levels at endpoint (Table 2) due to dilution with circulating blood in these 2 groups.

For substrate loading by intracoronary infusion, the stable isotopes were infused directly into the LAD via a 24-gauge BD Saf-T-catheter (Becton Dickinson, Sandy, UT) inserted just distal to the origin of the first branch. The intracoronary doses were adjusted to achieve 1.2 mM [2-^{13}C]lactate, 0.4 mM [2,4,6,8-^{13}C]octanoate and 0.4 mM [U–^{13}C]LCFA concentrations and were based upon the mean LV coronary artery flow per body weight calculated in preliminary immature pig experiments [17,18].

2.6. Metabolite analyses by NMR

Briefly, freeze-clamped hearts were ground into fine powder under liquid nitrogen and 0.5 mg further homogenized in 2.5 mL of a methanol/ddH_2O (1:0.25) mix. A 2:1 chloroform/ddH_2O mix was added to the homogenate, vortexed, and placed on ice for 10 min.