Differential effects of octanoate and heptanoate on myocardial metabolism during extracorporeal membrane oxygenation in an infant swine model

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Kajimoto M, Ledee DR, Olson AK, Isern NG, Des Rosiers C, Portman MA. Differential effects of octanoate and heptanoate on myocardial metabolism during extracorporeal membrane oxygenation in an infant swine model. Am J Physiol Heart Circ Physiol 309: H1157–H1165, 2015.—Nutritional energy support during extracorporeal membrane oxygenation (ECMO) should promote successful myocardial adaptation and eventual weaning from the ECMO circuit. Fatty acids (FAs) are a major myocardial energy source, and medium-chain FAs (MCFAs) are easily taken up by cell and mitochondria without membrane transporters. Odd-numbered MCFAs supply carbons to the citric acid cycle (CAC) via anaplerotic propionyl-CoA as well as acetyl-CoA, the predominant β-oxidation product for even-numbered MCFAs. Theoretically, this anaplerotic pathway enhances carbon entry into the CAC, and provides superior energy state and preservation of protein synthesis. We tested this hypothesis in an immature swine model undergoing ECMO. Fifteen male Yorkshire pigs (26–45 days old) with 8-h ECMO received either normal saline, heptanoate (odd-numbered MCFAs), or octanoate (even-numbered MCFAs) at 2.3 μmol·kg body wt·1·min⁻¹ as MCFAs systemically during ECMO (n = 5/group). The 13-carbon (¹³C) labeled substrates ([2-¹³C] lactate, [5,6,7-¹³C₃] heptanoate, and [U-¹³C₆] leucine) were systemically infused as metabolic markers for the final 60 min before left ventricular tissue extraction. Extracted tissues were analyzed for the ¹³C-labeled and absolute concentrations of metabolites by nuclear magnetic resonance and gas chromatography-mass spectrometry. Octanoate produced markedly higher myocardial citrate concentration, and led to a higher [ATP]-to-[ADP] ratio compared with other groups. Unexpectedly, octanoate and heptanoate increased the flux of propionyl-CoA relative to acetyl-CoA into the CAC compared with control. MCFAs promoted increases in leucine oxidation, but were not associated with a difference in protein synthesis rate. In conclusion, octanoate provides energetic advantages to the heart over heptanoate.

cardiac metabolism; extracorporeal circulation; fatty acids; pediatrics

NEW & NOTEWORTHY

Octanoate, an even-numbered medium-chain fatty acid, provides some energetic advantage for the heart, over odd-numbered heptanoate, during ventricular unloading by extracorporeal membrane oxygenation.

EXTRACORPOREAL MEMBRANE OXYGENATION (ECMO) is the most commonly used mechanical circulatory support system in infants and children with postoperative cardiopulmonary failure. Approximately 2–3% of infants undergoing congenital heart surgery require postoperative ECMO (18). However, mortality and morbidity remain high, with in-hospital mortality exceeding 50%. Duration of ECMO appears as a primary determinant of survival (5, 20). Inability to wean from the circuit due to persistent cardiac dysfunction prolongs the circuit run. Theoretically, cardiac dysfunction relates at least in part to imbalance between myocardial energy production and expenditure induced by cardiac injury and the ECMO-related inflammatory response (19, 26). ECMO reduces cardiac workload, myocardial oxygen consumption (MV\textsuperscript{O}_2), and thus ATP utilization. However, ATP production is dependent on maintenance of enzyme systems that direct carbon substrates toward and through the citric acid cycle (CAC), also known as tricarboxylic acid cycle, for oxidation (28). Our previous data showed no difference of [ATP]-to-[ADP] ratio and total CAC concentration, including citrate level between pre-ECMO and 8-h ECMO without nutritional support in animal studies (9, 11, 26).

Virtually all infants undergoing ECMO receive intravenous hyperalimentation consisting of a variety of substrates (6). Yet, few studies have evaluated substrate utilization to develop effective strategies for promoting successful myocardial adaptation during ECMO. Defining optimum nutritional support during ECMO is crucial in preventing protein breakdown and potential cardiac atrophy during prolonged ventricular unloading in infants. Our previous data suggested that medium-chain fatty acids (MCFAs) can potentially impact myocardial energy metabolism as accessible and flexible substrates during ECMO (11). Those data showed that the MCFA octanoate contributed approximately five times the carbon as acetyl-CoA to the CAC as did long-chain fatty acids (LCFAs) provided simultaneously under similar doses, MCFAs with chain lengths between 6 and 12 carbons are rapidly taken into cells and mitochondria. They do not use the rate-limiting membrane transporting systems required by LCFA (14). Thus, MCFA may provide superior energy balance and preservation of CAC intermediates in the heart supported by ECMO, than other substrates.

The MCFAs provide carbons to the CAC through two different mechanisms depending on their carbon chain number (Fig. 1). Even-numbered MCFAs such as octanoate undergo β-oxidation to acetyl-CoA, which then enters the CAC. Odd-numbered MCFAs similarly provide acetyl-CoA, but also yield propionyl-CoA as a final product of β-oxidation. Propionate or propionyl-CoA is efficiently converted through anaplerosis to the CAC intermediate, succinyl-CoA, in the myocardium. Previously, investigators have shown differential effects of even-numbered vs. odd-numbered MCFAs on the CAC intermediates in porcine myocardium (22). Some have suggested...
that enhanced propionate entry poses an energetic advantage for the heart (15) and have even embarked on clinical trials to test this premise in heart failure patients (ClinicalTrials.gov Identifier: NCT01787851). However, the influence of MCFA chain number on the CAC, myocardial energetics, and amino acid metabolism, particularly during ECMO in the immature heart, is unknown. The odd-numbered MCFA heptanoate contributes to the CAC via two pathways resulting in two acetyl-CoA and one propionyl-CoA molecule, whereas octanoate produces four acetyl-CoA molecules through β-oxidation (1). In the current study we determined if infusion with odd-numbered MCFA heptanoate during ECMO promotes greater anaplerosis and CAC intermediate accumulation than does octanoate. Furthermore, we evaluated the influence of these MCFA on pyruvate and amino acid oxidation, as well as protein synthesis.

MATERIALS AND METHODS

Animal protocol. All experimental procedures were approved by Seattle Children’s Institutional Animal Use and Care Committee. Fifteen male Yorkshire piglets (body wt 7.7–14.1 kg, age 26–45 days) were assigned randomly to three groups of five animals. All animals received ECMO for 8 h with continuous intravenous infusion of normal saline (Group-Con), heptanoate (Group-Hep), or octanoate (Group-Oct) as shown in Fig. 2A. Pigs were initially sedated with an intramuscular injection of ketamine (33 mg/kg) and xylazine (2 mg/kg) and were mechanically ventilated with an oxygen (40–50%) and isoflurane (1–2%) mixture, as described previously (8, 9, 11). After median sternotomy, recordings of aortic and coronary sinus return flow (Transonic Systems, Ithaca, NY) and left ventricular (LV) pressure (Millar Instruments, Houston, TX) were taken during the experiment, and a veno-arterial ECMO [a roller peristaltic pump console (Sarns 8000) and a hollow fiber membrane oxygenator (CXRX05SW; Terumo, Tokyo, Japan)] was established by central canulation via the ascending aorta and right atrium. Pump flow of ECMO was maintained at 100 ml·kg wt$^{-1}$·min$^{-1}$. Blood arterial and coronary venous blood samples were collected at multiple time points. Baseline data were obtained after administration of heparin. Blood samples were immediately centrifuged, and aliquots of plasma were stored at −80°C. 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). MV$\dot{O}_2$ was calculated from coronary venous flow and blood gas analysis. We maintained a pH of 7.35–7.45, an arterial PCO$_2$ of 35–45 mmHg, PO$_2$ of >100 mmHg, and a rectal temperature of 36–37.5°C during 8-h ECMO.

Nutritional supports with MCFA. Sodium heptanoate (C$_7$H$_{13}$NaO$_2$) and octanoate (C$_8$H$_{15}$NaO$_2$) were purchased from TCI America (Portland, OR). Heptanoate and octanoate are odd-numbered and even-numbered MCFA, respectively. Each of them was dissolved in 0.9% NaCl solution and was intravenously infused at 2.3 µmol·kg body wt$^{-1}$·min$^{-1}$ (6.6 ml/h, ~0.5 g fat·kg body wt$^{-1}$·day$^{-1}$) via extra jugular vein for 8 h. The dosage and infusion rate of these MCFA were decided in accordance with the recommended initial range of commercially available intravenous emulsion (clinical dosage of lipid nutrition: 0.5–3 g fat·kg body wt$^{-1}$·day$^{-1}$) (13, 21). NaCl solution (0.9%) was also infused at the same volume speed as control. The oxidation of one molecule of octanoate produces four acetyl-CoA, whereas that of heptanoate produces two acetyl-CoA and one propionyl-CoA, further metabolized to succinyl-CoA (Fig. 1). Arterial plasma free fatty acid (FA) concentration was measured at baseline (before starting ECMO), 4 h after starting ECMO, and at the endpoint of the experiment using commercial kits (Cayman, Ann Arbor, MI).

Metabolic analyses by nuclear magnetic resonance. Like the 13-carbon ($^{13}$C)-labeled substrates on heart, 1.2 mM [2-$^{13}$C]lactate, 0.2 mM [5,6,7-$^{13}$C]heptanoate, and 2 mM [U-$^{13}$C$_6$]leucine, which were purchased from Sigma-Aldrich, were delivered in the left anterior descending coronary artery for the final 60 min of the protocol. These intracoronary doses were based on the mean LV coronary artery flow per body weight calculated in preliminary immature pig experiments (23). $^{13}$C-nuclear magnetic resonance (NMR) was performed on the
Hep, Oct, or Octanoate (Group-Oct)]. The duration of membrane oxygenation (ECMO) [continuous iv infusion of normal saline groups were designed based on continuous iv nutrition during 8-h extracorporeal membrane oxygenation (ECMO) (16). The concentrations of the anaplerotic component to the CAC. 13C-NMR studies of CAC flux are based on the analysis of the steady-state 13C-glutamate spectrum. Glutamate is a suitable metabolite for 13C-NMR analysis because it is abundant in most mammalian tissue and is in rapid exchange with the CAC intermediate α-ketoglutarate (α-KG). The C4- and C5-glutamate are derived from acetyl-CoA, and carbon-1, -2, and -3 are derived from oxaloacetate (OAA) on each turn of the cycle. A relatively simple inspection of the intensities of carbon-5 and/or carbon-4 resonance spectra can also allow a direct estimation of the relative contributions of different substrates to acetyl-CoA entry in the CAC.

The anaplerotic contribution through propionyl-CoA from [5,6,7-13C3]heptanoate relative to acetyl-CoA entry was calculated by using several parameters obtained from the 13C-NMR spectrum. The general scheme for the labeling pattern of glutamate is shown in Fig. 1. Each oxidized labeled lactate molecule contributes a single labeled carbon to C5-glutamate, which produces an S spike within the spectrum. However, because lactate contributes only a portion of acetyl-CoA entering the CAC, we correct using the Fc lactate. In contrast, each molecule of propionyl-[U-13C3]CoA derived from [5,6,7-13C3]heptanoate is converted into succinyl-[1,2,3]- or [2,3,4-13C3]CoA and results in [1,2,3]- or [2,3,4-13C3]glutamate (17). 13C-labeling of C2-glutamate yields doublet (D23) complex and a quartet (Q). Thus, propionyl-CoA entry relative to acetyl-CoA entry can be estimated from peak areas as C2/D23 + Q/CS(S)/Fc lactate. For these studies recycling and relabeling of glutamate back through α-KG might conceivably occur. However, we considered this potential component as rather negligible, since it would require recycling uninterrupted and undiluted back to glutamate. Furthermore, the brief time required for steady-state labeling (1 h) in this model minimizes contribution through second-pass recycling.

Myocardial energy metabolites were measured by 1H-NMR spectra from extracted LV tissues as previously described (9, 11, 26). Collected spectra were analyzed using Chenomx software (version 7.7; Chenomx) with quantifications based on spectral intensities relative to 0.5 mM 2,2-dimethyl-2-silapentane-5-sulfonate, which was added as a spike to each sample. We also measured myocardial acetyl-CoA levels using a commercially available fluorometric assay kit according to the manufacturer’s protocols (BioVision, Milpitas, CA). Acetyl-CoA concentration was expressed as nanomole per gram of protein quantified from LV tissue lysates.

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Metabolic analyses by gas chromatography-mass spectrometry. Gas chromatography-mass spectrometry (GC-MS) was performed to measure the 13C enrichment and concentrations of CAC intermediates, pyruvate, lactate, and intracellular free leucine enrichment. GC-MS data are reported as the 13C-molar percent enrichment (MPE) and absolute quantity of each metabolite. Mass isotopomers of metabolites containing 1 to n 13C-labeled atoms were identified as Mi, with i = 1, 2, ..., n, and the total MPE of individual 13C-labeled mass isotopomers (Mi) of a given metabolite was calculated as follows: total MPE = ∑MPE/AM + ∑AMi.

Moreover, protein fractional synthesis rate (FSR) from leucine, which is an essential amino acid and cannot be synthesized by animals, was calculated as the ratio of 13C-leucine in the proteins and the free tissue fluid for 1 h following the methods of Jaleel et al. (7). A detailed description for the calculations of flux ratios has also been published previously (3, 9, 26).

**Immunoblotting.** Fifty micrograms of total protein extract from heart tissue were electrophoresed through 4.5% stacking and 10% running sodium dodecyl sulfate polyacrylamide gels and electroblotted on polyvinyl difluoride membranes. Equal protein loading of samples was determined by a protein assay (Bio-Rad, Hercules, CA) and confirmed by reversible protein stain kit for polyvinyl difluoride membranes (Thermo Scientific, Rockford, IL) and probing with anti-
bodies against α-tubulin (Santa Cruz Biotechnology, Dallas, TX). Membranes were probed overnight at 4°C with primary antibodies dissolved in phosphate-buffered saline-Tween 20 containing 5% milk or bovine serum albumin. The primary antibodies used in this study were pyruvate dehydrogenase (PDH), forkhead box O3 (FOXO3a), phospho-FOXO3a-Ser253, and phosphoprotein kinase B (Akt)-Ser473 obtained from Cell Signaling Technology (Danvers, MA), phospho-PDH-Ser203 obtained from Millipore (Billerica, MA), Akt and branch-chain ketoacid dehydrogenase kinase (BCKDK) obtained from Santa Cruz Biotechnology, branch-chain ketoacid dehydrogenase E1α (BCKDE1α) and phospho-BCKDE1α-Ser203 obtained from Bethyl Laboratories (Montgomery, TX), muscle-specific RING finger-1 (MuRF-1) obtained from GeneTex (Irvine, CA), and propionyl-CoA carboxylase α (PCCα) obtained from Abcam (Cambridge, MA). Blots were incubated at room temperature for 1 h with the appropriate secondary antibody conjugated to horseradish peroxidase. The blots were visualized with enhanced chemiluminescence after exposure to Kodak Biomax light ML-2 film. The densitometric intensities were determined using Image J analysis software (National Institutes of Health, Bethesda, MD). Western blots were repeated in triplicate to confirm the findings.

Statistical analyses. Reported values are means ± SE in text, Figs. 1–6, and Table 1. Significant differences from the baseline value for each group in Table 1 were estimated by a paired t-test. Other statistical analyses were performed by one-way ANOVA with Tukey’s post hoc test. Criterion for significance was P < 0.05 for all comparisons.

RESULTS

Fifteen pigs underwent 8-h ECMO with either normal saline, heptanoate, or octanoate randomly. There were no operative or technical complications in any groups.

Plasma FA levels. We measured plasma free FA levels before starting ECMO as a baseline, 4 h after starting ECMO, and just before completion of the labeled infusion as an endpoint. Free FA level in Group-Con was gradually dropped and just before completion of the labeled infusion as an endpoint. Free FA level in Group-Con was gradually dropped before starting ECMO as a baseline, 4 h after starting ECMO, and just before completion of the labeled infusion as an endpoint. Free FA level in Group-Oct occurred as a result of differences in carbon uptake among the three groups. The total sum of CAC intermediates was higher in the Group-Oct than the other two groups (210 ± 32, 174 ± 7, and 359 ± 63 nmol/g in Group-Con, -Hep, and -Oct, respectively, P < 0.05) (Fig. 3A). However, other CAC intermediates (α-KG, succinate, fumarate, malate, and OAA) were not different among the three groups. The total sum of CAC intermediates was higher in the Group-Oct than the other two groups (509 ± 23, 489 ± 45, and 696 ± 96 nmol/g in Group-Con, -Hep, and -Oct, respectively, P < 0.05) (Fig. 3B).

GC-MS for LV tissue showed that 13C-MPE of each CAC intermediate derived from exogenous 13C-labeled substrates was similar among the three groups (Fig. 3C). Despite the increased absolute value for citrate in the Group-Oct, we detected no differences in 13C-MPE among the three groups. This finding suggests that increased citrate accumulation in the Group-Oct occurred as a result of differences in carbon uptake among the groups throughout the protocol, as opposed to enhanced uptake of 13C label during the final 60 min.

Myocardial acetyl-CoA levels were not statistically different among the three groups in the present study (283.1 ± 25.5, 300.7 ± 51.2, and 313 ± 54.7 nmol/g protein wt in Group-Con, -Hep, and -Oct, respectively).

Octanoate increased cytosolic energy state. We also analyzed [ATP]/[ADP] as an index for phosphorylation potential. 1H-NMR showed that octanoate infusion elevated the [ATP]-to-[ADP] ratio (6.2 ± 0.6, 6.0 ± 0.6, and 7.8 ± 0.5 in Group-Con, -Hep, and -Oct, respectively, P < 0.05), implying a potentially high energy state (Fig. 3D).

MCFAs did not alter lactate oxidation. We analyzed lactate oxidation using Fc data supplied by 13C-NMR (Fig. 4, A–C). Fc from [13C]lactate was ∼30% without significant differences among the three groups. Phosphorylation of PDH by immunoblot was used as a surrogate for enzyme activity with no differences among the three groups (Fig. 4D). Fc from unlabeled substrates was reduced in Group-Oct, but it was not significantly different. GC-MS showed that all groups had similar absolute concentrations and 13C-MPE values for pyruvate and lactate in LV tissue at the end of the protocol (Fig. 4, A–C).

Table 1. Parameters of baseline just after cannulation for ECMO and end-point cardiac function

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<th>Baseline</th>
<th>Endpoint</th>
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<tr>
<td></td>
<td>Con (n = 5)</td>
<td>Hep (n = 5)</td>
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<tr>
<td>Hemoglobin, g/dl</td>
<td>9.9 ± 0.1</td>
<td>9.8 ± 0.5</td>
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<tr>
<td>HR, beats/min</td>
<td>106 ± 8</td>
<td>118 ± 5</td>
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<tr>
<td>LVSP, mmHg</td>
<td>87 ± 3</td>
<td>84 ± 3</td>
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<tr>
<td>LVEDP, mmHg</td>
<td>9 ± 1</td>
<td>8 ± 1</td>
</tr>
<tr>
<td>Pulse P, mmHg</td>
<td>38 ± 3</td>
<td>39 ± 2</td>
</tr>
<tr>
<td>CO, l/min</td>
<td>1.1 ± 0.1</td>
<td>1.1 ± 0.1</td>
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Values are means ± SE; n, no. of animals. There were no significant differences among 3 groups [continuous iv infusion of normal saline (Con), heptanoate (Hep), or octanoate (Oct)]. HR, heart rate; LVSP, left ventricular systolic blood pressure; LVEDP, left ventricular end-diastolic pressure; Pulse P, pulse pressure = (systolic-diastolic) systemic blood pressure; CO, cardiac output; ECMO, extracorporeal membrane oxygenation. *P < 0.05 vs. baseline.
These data demonstrated that nutritional support by MCFAs did not disturb lactate oxidation. Amino acid oxidation and protein turnover. The 13C-NMR data showed that the Fc of leucine was elevated in Group-Hep and -Oct compared with Group-Con (Fig. 4, B and C). GC-MS showed no difference in absolute tissue leucine or in MPE among the groups. 13C-enriched leucine in the extracted LV tissue was ~80% in all groups (Fig. 5A). The myocardial concentrations of other branched-chain amino acids (isoleucine and valine) by 1H-NMR were also similar for 13C enrichment and absolute concentrations among the three groups by GC-MS (Fig. 5, A and B). Calculated protein FSR from [13C]leucine by GC-MS ranged between 0.4 and 0.5%/h in all groups (Fig. 5D). Even- or odd-numbered MCFA did not make an impact on FSR from leucine. We also measured the protein expression levels associated with branched-chain amino acid catabolism (BCKDE1α and BCKDK) and protein degradation (Akt, FOXO3a, and MuRF-1) by Western blotting. These data showed no differences among the three groups (Fig. 5, E and F).

Octanoate modified heptanoate partitioning to the CAC. As noted, heptanoate is partitioned into acetyl-CoA and propionyl-CoA for entry into the CAC. We calculated anaplerotic contribution to the CAC intermediates by 13C-NMR. Initial analyses showed that anaplerotic contribution from propionyl-CoA relative to acetyl-CoA was significantly higher in Group-Oct compared with other groups (Fig. 6). However, we then accounted for the anaplerotic contribution derived from exogenous unlabeled heptanoate and estimated based on the final plasma concentration data of free FA in Group-Hep compared with average FA in the control group [control free FA]:

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\text{Total anaplerosis} = \text{uncorrected anaplerosis} \times \left\{1 + \left[\frac{(\text{plasma free FA}) - (\text{control free FA})}{0.2}\right]\right.\]

where 0.2 mM is the concentration of [13C]heptanoate in the coronary artery. After applying this correction, we found no difference between Group-Hep and -Oct (Fig. 6B). We also measured protein expression of PCC for propionyl-CoA conversion to succinyl-CoA through methylmalonyl-CoA. PCC was higher in Group-Hep and -Oct than Group-Con (Fig. 6C). Thus, long-term infusion of octanoate and heptanoate similarly increased anaplerotic propionyl-CoA entry compared with control.

**DISCUSSION**

Myocardial substrate utilization plays an important role in the recovery of cardiac function during ECMO support. Our previous studies showed that some ECMO conditions, particularly after ischemia reperfusion, disturb myocardial substrate utilization in juvenile piglets (4, 9, 11, 26). The heart unloaded by ECMO illustrates impairments in metabolic flexibility, and shows preference for utilization of MCFAs. Theoretically, deficits in myocardial ATP production can occur through depletion of CAC intermediates, thereby limiting delivery of reducing equivalents for oxidative phosphorylation. These deficits become most apparent during weaning from the ECMO circuit. While the intermediates in the first span of the CAC are
highly dependent on citrate formation from acetyl-CoA, the more distal span also depends on carbon entry via anaplerosis. In particular, studies in isolated perfused hearts showed that propionate can enter the CAC via conversion to succinyl-CoA, thereby supplying additional carbons and bypassing oxidative pathways (12, 27). However, propionate entry is highly dependent on the source of supply and the concentration. A prior study showed that odd-numbered MCFAs variably increased concentration of CAC intermediates after ischemia-reperfusion in porcine heart in vivo without affecting cardiac function (22). Surprisingly, in that study, heptanoate increased fumarate concentration, whereas the even-numbered MCFA increased succinate. The authors provided no detailed explanation for this seemingly paradoxical finding, which suggested that the even-numbered MCFA, which does not produce propionate, somehow promoted anaplerotic carbon entry at succinate.

In the current study, we reduced cardiac work and thus MVO₂ through clinically relevant unloading by ECMO. These conditions differ substantially from prior work with heptanoate in porcine heart (22), since we were not investigating the effects of ischemia and reperfusion. Under our experimental conditions, we expected that several hours of infusion with heptanoate would increase the concentration of the CAC intermediates through enhanced anaplerosis toward succinate. Elevated anaplerotic entry of [13C]propionate might be expected to increase the 13C-MPE of succinate relative to the 13C-MPE of α-KG. However, we found no differences in 13C-MPEs for these metabolites among the three experimental groups. Rather, we paradoxically found that prolonged octanoate infusion specifically increased absolute concentration of citrate without affecting the 13C-MPE of citrate. Although prolonged octanoate infusion also increased α-KG over hep-
tanoate infusion and control conditions (normal saline), these differences did not reach significance. Our inability to detect significant differences in \( \alpha \)-KG may be due to extremely low concentrations of this particular intermediate (\( \sim \)1/10 citrate concentration) and limitations in the sensitivity of our analyses. Constancy of the CAC intermediates MPE through all three protocols suggests that changes to the absolute concentrations are due to the prolonged MCFA infusion and not to differences in substrate handling during the \( ^{13} \)C-labeled substrate infusions.

The mechanism supporting specific increases in citrate concentration by octanoate and presumably \( \alpha \)-KG still requires clarification. The GC-MS analyses did not show differences in \( ^{13} \)C-MPE of succinate among the three groups. Thus, these data imply that the three protocols did not differ with respect to absolute flux from heptanoate-derived propionyl-CoA to succinate. However, the NMR spectra labeling patterns in our data demonstrated that prolonged MCFA infusion altered the mode of \( ^{13} \)C-heptanoate entry in the CAC. Heptanoate oxidation produces two acetyl-CoA molecules in addition to a propionyl-CoA. Both prior octanoate or heptanoate infusion modified \( ^{13} \)C entry in the CAC by increasing propionyl-CoA influx relative to entry via acetyl-CoA derived from multiple sources. Both MCFA infusions over the protocol period increased expression for regulatory PCC \( \alpha \), branched-chain ketoacid dehydrogenase E1\( \alpha \), BCKDK, BCKD kinase; Akt, protein kinase B; FOXO3a, forkhead box O3; MuRF-1, muscle-specific ring finger-1. Values are means ± SE; \( n = 5 \)/group.

Fig. 5. Concentration of CAC-related amino acids (A and B) and branched amino acid (C), protein fractional synthesis rate (D), and protein expression levels for branched amino acid catabolism (E) and protein degradation (F) from left ventricular tissues at the end of the protocol. These data were not significantly different among the 3 groups. FSR, protein fractional synthesis rate from exogenous leucine; BCKDE1\( \alpha \), branched-chain ketoacid dehydrogenase E1\( \alpha \); BCKDK, BCKD kinase; Akt, protein kinase B; FOXO3a, forkhead box O3; MuRF-1, muscle-specific ring finger-1. Values are means ± SE; \( n = 5 \)/group.
by altering the sources of acetyl-CoA provided to citrate synthetase. The NMR-based analyses showed that relative flux through PDH was similar among the three experimental groups as was phosphorylation state for this enzyme, a surrogate for activity. This is surprising, since octanoate under more normal cardiac working conditions and at similar concentration of infusion substrates inhibits flux through both pyruvate decarboxylation and/or pyruvate carboxylation (anaplerosis) (24, 29). Thus, our results may reflect the very low rates of MV$_{O2}$ prevalent in these hearts supported by ECMO. However, we did identify increased relative leucine oxidation in Group-Oct. In agreement with this observation, Buse et al. reported that octanoate stimulated leucine oxidation in the perfused rat heart (2). Leucine eventually catabolizes to acetyl-CoA and acetocacetate, thus emulating metabolism analogous to octanoate. Prior data in skeletal muscle show that octanoate promotion of leucine oxidation occurs through activation of BCKD (25). However, we found no difference in expression or phosphorylation of the principal enzymes responsible for this pathway. The finding that, with octanoate, neither free leucine concentrations nor leucine incorporation into protein decrease in the face of increased leucine oxidation suggests that net transmembrane transport of this amino acid increases. The precise mechanism affecting transport remains unclear, although our prior studies showed that pyruvate inhibits leucine entry (26).

We have previously outlined the limitations of this experimental model employing ECMO (4, 8–11, 26). We studied metabolic changes induced by ECMO supported by various MCFAs strategies. Because we sought to specifically evaluate octanoate vs. heptanoate, we did not include LCFAs in our emulsion. Thus the overall free FA supply to heart was lower than used in the typical nutritional support for infants receiving ECMO. The impact of either octanoate or heptanoate may be greater at higher dosages. In these experiments, we sought to segregate metabolic interventions during ventricular unloading by ECMO. Therefore, we did not superimpose injury before placement on ECMO, as occurs during most clinical scenarios. In these studies, we measured protein synthesis and leucine oxidation. Measurement of protein degradation and turnover was not technically feasible and remains a limitation. We did explore expression of enzymes such as MuRF-1, which regulate protein ubiquination and degradation, but found no difference between the groups.

Further future studies are necessary to determine the mechanism on the activation of anaplerotic propionyl-CoA entry to CAC by octanoate and if prolonged octanoate infusion during ECMO represents a benefit during weaning from ECMO and time-dependent metabolic changes.

In conclusion, these data demonstrate that octanoate specifically increases cardiac citrate concentration and cardiac energy state during ventricular unloading by ECMO compared with heptanoate. Both even- and odd-numbered MCFAs over long term can promote anaplerosis via propionyl-CoA; however, this is not a principal flux pathway compared with the oxidation pathway via acetyl-CoA under ECMO support. These alterations in citrate were accompanied by a slight increase in leucine oxidation, but without altering the protein synthesis rate. The increase in leucine oxidation may contribute to the observed increase in citrate, although other undefined mechanisms may play role. Overall, these metabolic shifts occurring after prolonged octanoate infusion were associated

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**Fig. 6. Anaplerotic contribution to CAC from propionyl-CoA.**

- **A**: representative spectra for C2-glutamate. C2 spectra showed large quartet spikes (Q) and D on C2 and -3 of glutamate in Group-Oct. Filled black bar, anaerobic contribution to CAC from $^{13}$C-heptanoate in Group-Oct; hatched bar, anaerobic contribution from $^{13}$C-heptanoate plus exogenous unlabeled heptanoate in Group-Hep. PPM = parts per million.
- **B**: anaerobic contribution to CAC from $^{13}$C-heptanoate in Group-Oct was significantly higher than that in Group-Con, and it was similar to anaerobic contribution on the basis of $^{13}$C-heptanoate plus exogenous unlabeled heptanoate in Group-Hep. Filled bar, anaerobic contribution in Group-Oct.
- **C**: protein expression levels of propionyl-CoA carboxylase α (PCCα) and α-Tubulin in Group-Oct was significantly higher in Group-Hep and -Oct than Group-Con. Values are means ± SE; n = 5/group. *P < 0.05 vs. Con.
with an increase in cytosolic energy state ([ATP]/[ADP]), suggesting an alteration in kinetic equilibrium within the CAC. However, the cardiac functional implications for these metabolic shifts need to be examined during conditions of pressure and volume reloading LV in future experiments.

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GRANTS
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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

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