**Background:** Extracorporeal membrane oxygenation (ECMO) provides a rescue for children with severe cardiac failure. It has previously been shown that triiodothyronine (T3) improves cardiac function by modulating pyruvate oxidation during weaning. This study focused on fatty acid (FA) metabolism modulated by T3 for weaning from ECMO after cardiac injury.

**Methods and Results:** Nineteen immature piglets (9.1–15.3 kg) were separated into 3 groups with ECMO (6.5 h) and wean: normal circulation (Group-C); transient coronary occlusion (10 min) for ischemia-reperfusion (IR) followed by ECMO (Group-IR); and IR with T3 supplementation (Group-IR-T3). 13-Carbon (13C)-labeled lactate, medium-chain and long-chain FAs, was infused as oxidative substrates. Substrate fractional contribution (FC) to the citric acid cycle was analyzed by 13C-nuclear magnetic resonance. ECMO depressed circulating T3 levels to 40% of the baseline at 4 h and were restored in Group-IR-T3. Group-IR decreased cardiac power, which was not fully restorable and 2 pigs were lost because of weaning failure. Group-IR also depressed FC-lactate, while the excellent contractile function and energy efficiency in Group-IR-T3 occurred along with a marked FC-lactate increase and [adenosine triphosphate]/[adenosine diphosphate] without either decreasing FC-FAs or elevating myocardial oxygen consumption over Group-C or -IR.

**Conclusions:** T3 releases inhibition of lactate oxidation following IR injury without impairing FA oxidation. These findings indicate that T3 depression during ECMO is maladaptive, and that restoring levels improves metabolic flux and enhances contractile function during weaning.  

**Key Words:** ECMO; Myocardial metabolism; Pediatric

---

**Editorial p 2836**

Accordingly, we have pursued a hypothesis that these metabolic disturbances impair ATP production and limit the ability of the heart on ECMO to re-establish contractile function. We further postulated that manipulation of substrate supply or hormonal readjustment such as T3 replacement would improve contractile function. We have followed a systematic experi-

Received July 29, 2014; revised manuscript received September 14, 2014; accepted September 16, 2014; released online October 28, 2014  Time for primary review: 20 days  Center for Developmental Therapeutics, Seattle Children’s Research Institute, Seattle, WA (M.K., D.R.L., C.X., H.K., M.A.P.); Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory, Richland, WA (N.G.I.); and Division of Cardiology, Department of Pediatrics, University of Washington, Seattle, WA (M.A.P.), USA  Mailing address: Michael A. Portman, MD, Center for Developmental Therapeutics, Seattle Children’s Research Institute, 1900 9th Ave, Seattle, WA 98101, USA  E-mail: michael.portman@seattlechildrens.org  ISSN-1346-9843  doi:10.1253/circj.CJ-14-0821  All rights are reserved to the Japanese Circulation Society. For permissions, please e-mail: cj@j-circ.or.jp
ECMO, and improved cardiac contractile function after ischemia, T3 supplementation promoted pyruvate flux through the citric acid cycle (CAC). Furthermore, successful weaning from ECMO required the flexibility to access both medium-chain and long-chain FAs (MCFAs and LCFAs) for oxidation and ATP production.

ECMO frequently provides support after ischemia-reperfusion (IR) injury, which is associated with cardiac surgery or arrest. We also demonstrated that both pyruvate loading and T3 supplementation promoted pyruvate flux through the CAC and improved cardiac contractile function after ischemia, which is associated with cardiopulmonary bypass. Finally, thyroid hormone re-established the ability of the heart to increase pyruvate oxidative flux during weaning from ECMO support for IR injury. Enhanced pyruvate flux occurred in conjunction with improved myocardial oxidative capacity and contractile function. The porcine studies were performed with the experiments using a high supraphysiological pyruvate concentration within the coronary arteries. Results from those studies also suggest that the heart relies heavily on an alternate undefined substrate during weaning. Free FAs, both MCFAs and LCFAs, are used clinically for nutritional support during ECMO, and at least theoretically provide efficient carbon substrate sources for ATP production. Therefore, we tested the hypothesis that thyroid hormone modulates pyruvate and FA oxidation under relatively physiological substrate conditions during weaning from ECMO.

Methods
Animal Models
Nineteen male Yorkshire piglets (body weight 9.1–15.3 kg, age 30–57 days) were used for the experiments. All experimental procedures were approved by Seattle Children’s Institutional Animal Use Committee. The piglets 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 with FiO2 40–60%, volume control 15 ml/kg, PEEP 3 cmH2O, respiratory rate 14–18/min and isoflurane 1–2% to maintain general anesthesia. Pigs were randomly divided into 3 study groups that underwent ECMO and weaning (Figure 1). Pigs in Group-IR and -IR-T3 were exposed to IR injury prior to ECMO. T3 (Sigma, St. Louis, MO, USA) was administrated at a bolus of 0.6 μg/kg and then continuous infusion was administrated at a rate of 0.2 μg · kg−1 · h−1 in Group-IR-T3. As control group, pigs did not receive either IR injury or T3 supplementation (Group-C). The ECMO duration was for 6.5 h, and the study endpoints of each group were at 1.5 h after weaning from ECMO. Plasma T3 concentrations were measured using commercial kits (Endocrine Technology, Newark, CA, USA).

After the performance of a median sternotomy, aortic flow and coronary flow probes (TS420; Transonic Systems Inc, Ithaca, NY, USA) and a left ventricular (LV) pressure catheter (Millar Instruments, Houston, TX, USA) were organized and then a 24-gauge BD Saf-T-Infusion catheter (Becton Dickinson, Sandy, UT, USA) was advanced just distal to the origin of the first branch from the left anterior descending coronary artery (LAD), as previously described. Coronary arterial occlusion was created by LAD ligation with a 4-0 monofilament sutures for 10 min, and then the suture was removed. Occlusion was visually confirmed by the change of color of the myocardium. We performed these experiments in immature piglets exposed to 10 min of ischemia created by LAD occlusion (Figure 2A). Our prior work showed that although coronary flow is fully re-established, this duration of ischemia causes high mortality due to cardiac contractile failure and marked contractile dysfunction in the survivors.

ECMO consisted of a roller peristaltic pump console (Sarns 8000; Terumo, Tokyo, Japan) and 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 2,000 units of heparin. The total prime volume was approximately 80 ml. A veno-arterial ECMO circuit was established by central cannulation into the ascending aorta and right atrium. Management during ECMO kept the pump flow rates at 80–100 ml · kg−1 · min−1. We maintained a pH of 7.35–7.45, an arterial pCO2 of 35–45 mmHg, an arterial pO2 of >120 mmHg, and a rectal temperature of 36–37.5°C. The ECMO duration time was 6.5 h. Perfusion flow of ECMO was decreased gradually for 30 min and then ECMO was weaned. The animals did not receive any blood transfusions or inotropic or vasoactive drugs.

Myocardial Oxygen Consumption and Cardiac Parameters
Cardiac output (CO) was measured by aortic flow meter directly. Cardiac power [Watt] was calculated as the mean arterial pressure ×CO/451, where mean arterial pressure=(systolic pressure−2×diastolic pressure)/3. Myocardial oxygen consumption (MVO2) [μmol · min−1 · g−1] was calculated from the coronary flow and the difference in oxygen content of systemic artery and coronary venous. Cardiac energy efficiency [%] was calculated as the cardiac power/MVO2. For this formula, MVO2 was converted to Joules [J/min] using the conversion of 1 μmol O2=0.4478 J, as described by Suga.

Evaluation of Myocardial Injury Levels
As an index of ischemic myocardial injury, the plasma cardiac
Triiodothyronine Improves Weaning From ECMO

Metabolic Analysis
We examined substrate utilization to the CAC using 13-Carbon (13C)-labeled substrates and nuclear magnetic resonance (NMR) analysis, as previously described. We analyzed the central portion of the myocardial ischemic injured zone in strict concordance with the perfused area of 13C-substrates for these metabolic studies. [2,4,6,8-13C]octanoate, MCFA (Sigma St. Louis, MO, USA) and [U-13C]LCFAs (Cambridge Isotope Laboratories, Andover, MA, USA) were used as the metabolic markers. [U-13C]LCFAs consist of palmitic acid (45–55%), palmitoleic acid (10–15%), oleic acid (20–30%) and linoleic acid (10–15%). Each 13C-labeled substrate labels 13C-labeled acetyl-CoA differently, and the pattern is reflected by the pattern of 13C-glutamate in the NMR spectrum.

Infusion of labeled Substrates
The 13C-labeled substrates were infused directly into the LAD for the final 60 min of the protocol. Based on coronary artery flow, intracoronary concentrations of infusing labeled substrates were adjusted to 1.2 mmol/L [2-13C]lactate, 0.1 mmol/L [2,4,6,8-13C]octanoate and 0.1 mmol/L [U-13C]LCFAs to closely match the physiological concentration. Immediately upon completion of the 13C-labeled substrates infusion, portions of LV myocardial tissue perfused by the LAD were quickly freeze-clamped and stored at −80°C for later extraction.

NMR
13C-NMR was performed on the myocardium for the determination of specific carbon glutamate labeling. We used methanol extraction for the LV tissue, and NMR spectra were acquired on a Varian Direct Drive (VNMRS) 600 MHz spectrometer (Varian Inc, Palo Alto, CA, USA), as previously described. The labeled carbon resonances (C3–C5) of glutamate were integrated using commercial software (NUTS; Acorn NMR, Livermore, CA, USA). The individual integral values were used as starting parameters for the CAC analysis-fitting algorithm, tcaCALC, kindly provided by Drs C. R. Malloy and F. M. Jeffrey. This algorithm provided the fractional enrichment for each substrate to the acetyl-CoA pool entering the CAC. Energy metabolites, lactate and pyruvate, were also measured by 1H-NMR spectra from LV tissues prepared by using methanol, as previously described. Collected spectra were analyzed using Chenomx 7.6 software (Edmonton, Alberta, Canada), with quantifications based on spectral intensities relative to 0.5 mmol/L 2,2-dimethyl-2-silapentane-5-sulfonate, which was added as a spike to each sample.

Western Blotting
Immunoblot analyses were used to evaluate the expression of key proteins regulating substrate oxidation. Fifty micrograms of total protein extract from the heart tissue were electrophoresed through 4.5% stacking and 10% running SDS-polyacrylamide gels, and electroblotted onto PDVF-plus membranes. Membranes were blocked for 1 h at room temperature with 5% non-fat milk in Tris-buffered saline plus Tween-20 (TBST: 10 mmol/L Tris-HCl, pH 7.5, 150 mmol/L NaCl, and 0.05% Tween-20). Equal protein loading of samples was determined by a protein assay (BioRad, Hercules, CA, USA) and confirmed by using a reversible protein stain kit for PVDF membranes (Thermo Scientific, Rockford, IL, USA). The primary antibodies used in this study were 5’ adenosine monophosphate-activated protein kinase α (AMPKα), phospho-AMPKα-Thr172, acetyl-CoA carboxylase (ACC), phospho-ACC-Ser79 and pyruvate dehydrogenase (PDH), obtained from Cell Signaling Technology (Danvers, MA, USA), and phospho-PDH-Ser293, which was obtained from Millipore (Billerica, MA, USA). 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, USA). Western blots were repeated in triplicate to confirm the findings.
I concentrations in Group-IR and Group-IR-T3 were 0.45±0.05 and 0.49±0.05 ng/ml, respectively, at 4 h after starting ECMO, and were significantly higher than that in Group-C (0.21±0.03 ng/ml, P<0.01, Figure 2B).

Plasma T3 Levels
We measured plasma T3 levels before ischemic injury and ECMO as a baseline, at 1 and 4 h after starting ECMO, and just before completion of the labeled infusion as an endpoint. The T3 levels in Group-C and Group-IR gradually dropped off after starting ECMO, whereas they were maintained at baseline levels until the end of the protocol for Group-IR-T3 (Figure 3).

Cardiac Function
Baseline hemodynamics were not significantly different among the 3 groups (Table 1). During ECMO, the LV end-diastolic pressure significantly dropped below 4 mmHg in all animals. Table 2 showed parameters of cardiac function relative to baseline at the endpoint of each protocol. Hemoglobin, heart rate and mean systemic blood pressure at the endpoint of the protocol were not significantly different among the 3 groups. As noted in previous publications, hemoglobin dropped slightly due to the lack of blood pump prime, and ranged it between 6.7 and 8.8 g/dl in all animals. IR injury significantly decreased this index, with reversal by T3 supplementation. Therefore, the data, which included only survivors, suggests that T3 at least partially reverses abnormalities in high energy phosphate metabolism induced by IR injury. The [reduced nicotinamide adenine dinucleotide: NADH]/[oxidized nicotinamide adenine dinucleotide: NAD] and [reduced nicotinamide adenine dinucleotide phosphate: NADPH]/[oxidized nicotinamide adenine dinucleotide phosphate: NADP] data are indicators of a cel-

Table 1. Parameters of Baseline Cardiac Function Just After Cannulation for ECMO

<table>
<thead>
<tr>
<th></th>
<th>Group-C (n=5)</th>
<th>Group-IR (n=6)</th>
<th>Group-IR-T3 (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>9.3±0.9</td>
<td>9.1±0.3</td>
<td>9.5±0.5</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>117±4</td>
<td>105±5</td>
<td>111±8</td>
</tr>
<tr>
<td>Mean SBP (mmHg)</td>
<td>57±3</td>
<td>63±3</td>
<td>61±3</td>
</tr>
<tr>
<td>LVEDP (mmHg)</td>
<td>7±1</td>
<td>7±1</td>
<td>6±1</td>
</tr>
<tr>
<td>CO (L/min)</td>
<td>1.14±0.08</td>
<td>1.05±0.13</td>
<td>1.04±0.04</td>
</tr>
<tr>
<td>Power (Watt)</td>
<td>0.20±0.01</td>
<td>0.21±0.04</td>
<td>0.20±0.01</td>
</tr>
<tr>
<td>MVO2 (μmol·min⁻¹·g⁻¹)</td>
<td>3.79±0.28</td>
<td>4.36±0.43</td>
<td>3.61±0.43</td>
</tr>
<tr>
<td>Efficiency (%)</td>
<td>24.9±3.2</td>
<td>21.2±4.3</td>
<td>21.6±1.5</td>
</tr>
</tbody>
</table>

There were no significant differences among groups. Values are presented as mean±SE.

CO, cardiac output; ECMO, extracorporeal membrane oxygenation; Group-C, control group; Group-IR, Ischemia-reperfusion group; Group-IR-T3, T3 treatment group; HR, heart rate; LVEDP, left ventricular end-diastolic pressure; MVO2, myocardial oxygen consumption; SBP, systemic blood pressure.

Figure 3. Plasma triiodothyronine (T3) levels. In Group-C and Group-IR, plasma T3 levels were decreased gradually after starting extracorporeal membrane oxygenation (ECMO). T3 supplementation significantly prevented the reduction of total T3 levels. Values are presented as mean±SE; n=5–6 per group. *P<0.05 vs. Group-C and Group-IR. Group-C, control group; Group-IR, Ischemia-reperfusion injury group.

Statistical Analyses
Reported values are mean±standard error (SE) in figures, text, and tables. All data were evaluated by one-way ANOVA with Tukey’s post-hoc test. The criterion for significance was P<0.05 for all comparisons.

Results
Survivors and Validation of Ischemic Injury
We directly observed color change as demarcated in Figure 2A during coronary constriction. Survival was determined by the ability to wean from the ECMO and maintain circulation without support for 1 h. All animals survived except for 2 in the IR group, leaving Control 5/5, IR 6/8 and IR-T3 6/6 for further analyses. Further validation of ischemic injury was provided by plasma troponin-I concentrations, which increased significantly in our ischemic groups. Plasma cardiac troponin-

High Energy Phosphate Metabolism
We used 1H-NMR to determine myocardial energy metabolite ratios following weaning and identified modest though not significant decreases in [Phosphocreatine; PCr]/[ATP] in survivors (Figure 4A). These changes were partially reversed with T3 supplementation. This ratio can be increased by depletion of the ATP pool. We therefore also analyzed [ATP]/[adenosine diphosphate: ADP]; another index for phosphorylation potential (Figure 4B). IR injury significantly decreased this index, with reversal by T3 supplementation. Therefore, the data, which included only survivors, suggests that T3 at least partially reverses abnormalities in high energy phosphate metabolism induced by IR injury. The [reduced nicotinamide adenine dinucleotide: NADH]/[oxidized nicotinamide adenine dinucleotide: NAD] and [reduced nicotinamide adenine dinucleotide phosphate: NADPH]/[oxidized nicotinamide adenine dinucleotide phosphate: NADP] data are indicators of a cel-
Triiodothyronine Improves Weaning From ECMO

2871

Table 2. Parameters of Cardiac Function at the End of the Protocol

<table>
<thead>
<tr>
<th></th>
<th>Group-C (n=5)</th>
<th>Group-IR (n=6)</th>
<th>Group-IR-T3 (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survival</td>
<td>5/5</td>
<td>6/8</td>
<td>6/6</td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>7.4±0.8</td>
<td>7.0±0.4</td>
<td>7.5±0.5</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>129±10</td>
<td>133±7</td>
<td>133±8</td>
</tr>
<tr>
<td>Mean SBP (mmHg)</td>
<td>49±3</td>
<td>51±3</td>
<td>58±6</td>
</tr>
<tr>
<td>LVEDP (mmHg)</td>
<td>7±1</td>
<td>9±1</td>
<td>6±1†</td>
</tr>
<tr>
<td>CO (% of baseline)</td>
<td>89.7±7.9</td>
<td>85.3±7.7</td>
<td>114.8±9.2†</td>
</tr>
<tr>
<td>Power (% of baseline)</td>
<td>81.9±10.3</td>
<td>71.9±12.4</td>
<td>117.9±9.5*†</td>
</tr>
<tr>
<td>MVO2 (μmol · min⁻¹ · g⁻¹)</td>
<td>3.76±0.43</td>
<td>2.72±0.41</td>
<td>2.93±0.70</td>
</tr>
<tr>
<td>Efficiency (%)</td>
<td>23.3±5.9</td>
<td>18.9±3.0</td>
<td>29.3±2.7†</td>
</tr>
</tbody>
</table>

Values are presented as mean ± SE. *P<0.05 vs. Group-C, †P<0.05 vs. Group-IR. Abbreviations as in Table 1.

ATP, adenosine triphosphate; ADP, adenosine diphosphate; NADH, reduced nicotinamide adenine dinucleotide; NAD, oxidized nicotinamide adenine dinucleotide; NADPH, reduced nicotinamide adenine dinucleotide phosphate; NADP+, oxidized nicotinamide adenine dinucleotide phosphate; Group-C, control group; Group-IR, ischemia-reperfusion group; Group-IR-T3, T3 treatment group; PCr, Phosphocreatine; T3, triiodothyronine.

Figure 4. Energy metabolites by 1H-NMR. Myocardial [PCr]/[ATP] and [ATP]/[ADP], and [NADH]/[NAD⁺] and [NADPH]/[NADP⁺] changed among the 3 groups, respectively (A-D). Group-IR demonstrated significantly lower [ATP]/[ADP] and [NADPH]/[NADP⁺] than Group-C, and T3 improved [ATP]/[ADP]. Ischemic injury increased the lactate concentration of left ventricular tissues compared with non-ischemia, but this did not reach statistical significance (E). The pyruvate concentration of left ventricular tissue was not statistically different among the 3 groups (F). The ratio of [Pyruvate]/[Lactate] in Group-IR and Group-IR-T3 was significantly decreased compared with Group-C (G). Values are presented as mean ± SE; n=5–6 per group. *P<0.05, †P<0.01 vs. Group-C. ATP, adenosine triphosphate; ADP, adenosine diphosphate; NADH, reduced nicotinamide adenine dinucleotide; NAD, oxidized nicotinamide adenine dinucleotide; NADPH, reduced nicotinamide adenine dinucleotide phosphate; NADP+, oxidized nicotinamide adenine dinucleotide phosphate; Group-C, control group; Group-IR, ischemia-reperfusion group; Group-IR-T3, T3 treatment group; PCr, Phosphocreatine; T3, triiodothyronine.

Luminor redox state (Figures 4C,D). Ischemia-reperfusion decreases these ratios, though only reaching significance for [NADPH]/[NADP⁺]. Ischemic injury tended to increase lactate concentration of LV tissues compared with non-ischemia, and T3 did not influence in lactate and pyruvate accumulations (Figures 4E,F). The [Pyruvate]/[Lactate] ratio (Figure 4G), another surrogate for redox state, decreases with ischemia, with no reversal by T3.
Substrate Metabolism

The $^{13}$C-NMR analyses provide fractional contributions (FCs) of acetyl-CoA to the CAC from 3 labeled substrates and a fourth-but-undefined unlabeled component. Typical and representative $^{13}$C-spectra identifying glutamate peaks are shown in Figures 5A and B. In all studies, $^{13}$C-enrichment was modest, and the unlabeled fraction represented the majority of oxidized substrate. Ischemia decreased FC from $^{13}$C-labeled substrates (32.6±2.5%) compared to the control (48.0±5.1%, P<0.05), and this was reversed by T3 supplementation (Figure 5C). The FC-lactate in Group-IR (10.3±1.0%) was lower than that in Group-C (7.7±0.7%); however, that did not reach statistical difference (P=0.08). Furthermore, FC-lactate was significantly higher in the T3-supplemented group (near 70% compared to Group-IR, P<0.05). The FC for $^{13}$C-labeled substrates can be influenced by their uptake as well as the size of the unlabeled pool in the myocardium. Absolute lactate levels and pyruvate detected by $^1$H-NMR were not altered by the protocol, suggesting that the substrate pool size was not responsible for these alterations in FC from labeled substrate.
(Figures 4E,F). Of note, our prior studies showed that T3 decreased total lactate and pyruvate concentration. However, the discrepancy might be related to higher pyruvate loading with supraphysiological concentrations in those experiments.\textsuperscript{15}

We also analyzed the relative contribution among the $^{13}$C-labeled substrates (Figure 5D). Lactate contribution was higher in Group-IR-T3 than in the other groups, but it did not reach significant difference ($P=0.08$). The MCFA, octanoate, provided the majority of the contribution from $^{13}$C-labeled substrate through all 3 protocols. Interestingly, T3 significantly decreased FC-MCFA and tended to increase FC-LCFAs; however, contribution of total FAs (sum of MCFA and LCFAs) was not significantly different among the 3 groups. Anaplerosis can replenish the CAC intermediates. However, these pathways produce futile cycling as they bypass substrate oxidation for entry into the CAC. We previously studied a major route of anaplerosis, pyruvate carboxylation, and found that its contribution, relative to pyruvate carboxylation, was not altered by IR or short-term T3 supplementation.\textsuperscript{9} In the current study, the labeling strategy did not permit specific analyses for pyruvate carboxylation. However, the tcaCALC algorithm yields data for total anaplerotic carbon contribution relative to the citric synthetase flux. Although we identified a considerable contribution to the CAC in each group, there were no significant differences (Figure 5E).

**Immunoblot Analyses**

We measured protein expression levels of phospho-ACC (inactive form of ACC), which inhibits the $\beta$-oxidation of FAs in the mitochondria; phospho-AMPK$\alpha$, which activates the cellular uptake of glucose through glucose transporter 4 and the $\beta$-oxidation of FAs through the phosphorylation of ACC, and phospho-PDH (inactivate form of PDH), which inhibits pyruvate decarboxylation as myocardial metabolism-related proteins (Figure 6). These protein levels were similar among the 3 groups, except for the increase of the phospho-PDH relative to total PDH caused by ischemic injury. Inactivation of PDH in Group-IR corresponded to the results of $^{13}$C-NMR, showing lower FC-lactate in Group-IR than that in Group-C.

**Discussion**

Our study shows that thyroid hormone repletion during ECMO modifies cardiac substrate oxidation by promoting the relative contribution of lactate to the CAC and thereby providing a potential mechanism for elevating contractile function during weaning. We cannot define in these experiments whether increased contractile function stems from the ischemic, border or non-ischemic zones. However, prior studies have in other experimental models shown that T3 provided acutely in similar doses promotes contractile function in IR but not in uninjured myocardium.\textsuperscript{30} In addition, our previous study assessed the effectiveness of T3 in the uninjured heart during ECMO.\textsuperscript{11} This data demonstrated that T3 markedly increased lactate oxidation in the uninjured heart. Unfortunately, cardiac performances were not clearly expressed under supported by ECMO. We cannot exclude the potential effects that uninjured myocardium contributes to the improvement of cardiac work compared with injured myocardium. To summarize, our previous and present results suggest that T3 improves cardiac work, and that activation of carbohydrate oxidation in both ischemic injured and uninjured myocardium is one potential mechanism of this effect.

Results from our current study support data from our previous study that related to thyroid hormone regulation of pyruvate decarboxylation.\textsuperscript{9,15} In those studies, we used supraphysiological doses of pyruvate without providing FAs in the coronary artery in order to maximize flux through the pyruvate decarboxylation pathway. In the current study, we confirmed the effect on pyruvate decarboxylation using more physiological concentrations for substrates, which are typically oxidized by the immature heart. Although FAs provide superior ATP production per carbon compared to carbohydrates, the latter are more oxygen efficient for ATP synthesis. Improved efficiency for ATP synthesis is not really relevant under conditions with ample O$_2$ supply. In this model, T3 improves effi-
Circulation Journal Vol.78, December 2014

from carbohydrates to FAs after birth. In order to emulate the clinical scenario. The immature porcine myocardium resembles that found in isolated rat myocardium, we could not document similar changes in activation through the phosphorylation of PDH. Furthermore, we also found no change in the phosphorylation of ACC, a primary regulator of free FA flux. Thus, the mechanisms driving these relative flux changes still require clarification in vivo.

We supplied both MCFAs and LCFAs in these experiments in order to emulate the clinical scenario. The immature porcine heart rapidly transitions the primary oxidative substrates from carbohydrates to FAs after birth. These studies confirm heavy reliance on these FAs, as they accounted for the majority of labeled substrate entering the CAC as acetyl-CoA. As noted in other experiments performed in multiple models, IR appears to impair overall FA oxidation. Our prior studies in isolated perfused rat heart showed that T3 infusion abruptly increases FC-LCFAs. However, this effect was reversed by epinephrine, which also enhanced T3 promotion of lactate oxidation. In the current study, we did not use adrenergic or any inotropic stimulation, although inotropic agents are frequently used clinically. Conceivably, ambient levels of circulating catecholamines following IR are enough to emulate the experiments performed in the isolated rat heart and modify the T3 affect on FA metabolism. In this study, lactate provided the principal source of acetyl-CoA produced by pyruvate decarboxylation. Lactate, in addition to FAs, generally supplies the overwhelming majority of oxidative substrate to the heart, while glucose provides a minor contribution. These substrates are also typically provided as nutritional support to infants during ECMO. Our data show that T3 increases FC from 13C-lactate by nearly 70%. The NMR resolution in our experiments showed that few pigs survived to weaning after longer periods of coronary occlusion. We documented ischemia through direct observation, and noted marked increases in troponin leak, thereby validating ischemic injury. Accordingly, our observed changes in cardiac function and metabolism caused by T3 appear modest, although some were statistically significant. Our study design did introduce some bias by the exclusion of non-survivors. Obviously, we could not measure function or metabolism in the two piglets which could not maintain circulation and wean. We chose not to assume a contractile functional status for these piglets, but recognize that any incorporation of their data would enhance the functional differences between Group-IR and Group-IR-T3. Second, MVO2 was calculated using total coronary flow reflected by sidestream return to coronary sinus as a simple technique, because direct measurement of coronary flow by using flow pad or probe potentially might change the flow in a subtle way in a small piglet heart. As noted previously, inotropic agents are used in the majority of patients supported by ECMO. The basis for their utilization is not well substantiated and remains mainly theoretical. A recent clinical trial in infants undergoing cardiopulmonary bypass showed that T3 supplementation, similar to that used in our experiments, reduced postoperative inotropic use. Accordingly, we did not feel it was necessary to add an inotropic agent to our protocol.

In summary, we have noted that ischemia-reperfusion in this immature pig model alters substrate metabolism, causing impairment in high energy phosphate status. T3 supplementation reverses some of these impairments, in part, by increasing relative flux through PDH without substantial disturbance of FA metabolism, and facilitates weaning from ECMO following cardiac injury in an immature swine model. These data support the notion that metabolic manipulation by thyroid hormone can improve weaning from ECMO. Although these experiments evaluated responses in immature myocardium, adults also undergo ECMO support. The relevance of these studies for adults undergoing ECMO can be a subject for future studies.

Acknowledgments

This work was supported by the National Institutes of Health [R01HL60666 to M.A.P.]. A portion of the research was performed using the Environmental Molecular Sciences Laboratory, a national scientific user facility sponsored by the Department of Energy’s Office of Biological and Environmental Research and is located at Pacific Northwest National Laboratory.

2874 KAJIMOTO M et al.
Disclosures

None.

References