Myocardial oxidative metabolism and protein synthesis during mechanical circulatory support by extracorporeal membrane oxygenation

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EXTRACORPOREAL MEMBRANE OXYGENATION (ECMO) is the principal form of mechanical circulatory support for pediatric patients suffering from acute cardiac decompensation after surgical procedures for congenital heart defects. ECMO is also instituted for heart failure in children from other causes such as sudden cardiac arrest or acute myocarditis (13, 17). Veno-arterial ECMO provides biventricular unloading by redirecting systemic venous return into a mechanical circuit where the blood is oxygenated and returned to the aorta. The ECMO circuit contains a pump that supports systemic blood flow and mean arterial pressure but reduces aortic pulsatility (13). Mortality either during or immediately after ECMO remains high, especially for infants and children with complex congenital heart disease (33). ECMO duration for cardiac indications is usually 24 to 48 h and is intended as a short-term bridge to recovery (13). When used for longer timer periods, the risks for mortality and severe morbidities including stroke and renal failure increase substantially. Therefore, early recovery from the antecedent insult and complete weaning and separation from the support circuit represent the principal goals of ECMO therapy for infants and children. However, ECMO frequently induces a cardiac stun syndrome (21). Stunng refers to sudden exacerbation of myocardial dysfunction observed in infants (21) and in animal models (31) within a few hours after instituting ECMO. The cardiac stun syndrome prevents or delays weaning from the support circuit and adversely affects survival.

Some investigators have hypothesized that inflammatory cytokines mediate or at least play an important role in the pathogenesis of ECMO-related cardiac stun. ECMO and closely related cardiopulmonary bypass procedures induce marked elevations in circulating plasma levels of multiple proinflammatory cytokines (22). Fairly modest cytokine elevations, relative to those associated with ECMO, yield metabolic abnormalities such as reductions in insulin sensitivity and shifts in substrate utilization (19). In turn, these metabolic abnormalities could affect the efficiency of cardiac ATP production and utilization, and therefore further impair the myocardial functional response to stress. Catabolic processes induced by inflammation could also modify cardiac protein balance, reduce myocardial mass, and further limit the ability of the heart to successfully wean from ECMO support. Although whole body studies in infants have documented elevations in protein turnover and amino acid oxidation during ECMO (3, 18, 34, 39), heart-specific modifications in substrate...
metabolism and protein synthesis have not been evaluated (3, 18, 34, 39). Thus we do not know if shifts occur in cardiac metabolism during ECMO or whether they represent potential targets for prevention of the cardiac stun syndrome.

Therefore, we formulated a hypothesis that ECMO alters amino acid oxidative metabolism and myocardial protein synthesis. To test this hypothesis we used a translational piglet model (32), which emulates ECMO in infants and children in these experiments, and pursued the fate of leucine delivered into the coronary artery, as a template for amino acid metabolism. The majority of leucine entering cardiomyocytes is either incorporated into protein or metabolized to acetoacetate and acetyl-CoA. These latter metabolites enter the citric acid cycle (CAC) and participate in oxidation, which supplies energy for ATP production. Accordingly, we measured leucine contribution to these pathways using \[^{13}\text{C}\]-Carbon (\[^{13}\text{C}\])-isotopomer analyses by both gas chromatography-mass spectrometry (GCMS) and nuclear magnetic resonance spectrometry (NMR). \[^{13}\text{C}\]-labeled pyruvate was included as a substrate in some experiments to serve as an alternate caloric source as well as a reference for oxidation by NMR; we have previously described the inotropic and metabolic effects of pyruvate in immature swine hearts (20, 27).

MATERIALS AND METHODS

Model

We used immature mixed breed Yorkshire male piglets in these experiments. Studies were approved by the Seattle Children’s Research Institute Animal Care and Use Committee and adhered to both the American Physiological Society’s Guiding Principles in the Care and Use of Animals and the National Institute of Health’s Guide for the Care and Use of Laboratory Animals.

In preliminary studies, we determined that mechanical circulatory support caused marked hemodilution in piglets weighing less than 7 kg. Hemodilution could be avoided by priming the support circuit with donor pig blood; however, this frequently caused devastating transfusion reactions. Pig cross-matching for blood priming of the pump is complicated by numerous serotypes (35). Accordingly, we set a lower threshold of 7 kg for pigs in this study. Male piglets between 27 and 41 days of age and weighing between 7.8 and 14.5 kg were prepared essentially as previously described (27). These pigs were beyond the neonatal age, but still immature and undergoing rapid cardiac growth. This developmental state approximates that for the human infants and neonatal pigs a rapid rise in plasma levels for some cytokines occurs over 6 h, followed by a plateau phase for at least 2 h in human infants and neonatal pigs (3, 22, 25, 28). Systemic hormonal and metabolic responses are attributed at least in part to the surge in cytokines. The cardiac stun phenomenon is concomitantly observed within this 8-h time frame. Therefore, we selected 8-h duration for all experimental groups as an appropriate time period for initial studies of metabolism and protein synthesis in this ECMO model.

Substrate Delivery

\[^{13}\text{C}_6\,^{15}\text{N}\]-L-leucine (Sigma-Aldrich, St. Louis, MO) alone or with sodium-[2-\[^{13}\text{C}\]]-pyruvate (Sigma-Aldrich) was delivered by intracoronary infusion. The direct coronary infusion eliminated potential issues with systemic infusion relevant to hemodilution and differences in vascular volume between control pigs and those on mechanical circulatory support. We based the target concentration of leucine (3.7 mM) on prior studies by Chua et al. (7–10), which were performed in isolated perfused working rat heart. Our objective was to determine the fractional contribution of leucine (FcLeucine) to the CAC relative to another typically oxidized substrate. We previously evaluated pyruvate oxidation and anaplerotic contribution to the CAC in piglets (8, 26, 27). Those experiments showed that intracoronary pyruvate concentrations near 8 mM provided adequate NMR signal for spectral analyses. Because leucine provides twice as many carbons as pyruvate, we then adjusted the dose in mM to a near 1-to-2 molar ratio for labeled leucine and pyruvate. Accordingly, the target concentration was 3.7 mM for \[^{13}\text{C}_6\,^{15}\text{N}\]-L-leucine and 7.4 mM for sodium-[2-\[^{13}\text{C}\]]-pyruvate.

Because substrates were delivered directly into the coronary artery, we were unable to sample downstream to confirm the actual concentrations delivered. However, the dose per milligram tissue was confirmed by heart wet weight after completion of the experiments. Studies in working and Langendorff perfused rat hearts have shown that oxidation of leucine supplied at either 1 or 5 mM achieves steady-state within a few minutes (15). We performed preliminary studies using a 2-h infusion of labeled leucine in two pigs under ECMO (data not shown). In these studies we confirmed that isotopic enrichment of protein and the cytosolic leucine pool as well as the CAC intermediates was similar to that obtained with a 1-h infusion. Using this as evidence for steady-state, we set the intracoronary substrate infusion duration for the protocol to 1 h.

ECMO Duration

ECMO initiates a massive systemic inflammatory response. In human infants and neonatal pigs a rapid rise in plasma levels for multiple cytokines occurs over 6 h, followed by a plateau phase for at least 2 h in human infants and neonatal pigs (3, 22, 25, 28). Systemic hormonal and metabolic responses are attributed at least in part to the surge in cytokines. The cardiac stun phenomenon is concomitantly observed within this 8-h time frame. Therefore, we selected 8-h duration for all experimental groups as an appropriate time period for initial studies of metabolism and protein synthesis in this ECMO model.
Protocols

We had four experimental groups based on loading status and substrate(s) provided in the coronary infusate. The piglets with normal circulation (not undergoing ECMO) were termed the LOAD group and provided a control for comparison with those undergoing ECMO. Each animal received infusion of [13C6,15N]-L-leucine, and half the piglets also received sodium-[2,3-13C]-pyruvate, so the four groups are as follows: LOAD-LP (normal circulation, leucine and pyruvate; n = 6), LOAD-L (normal circulation, leucine only; n = 5), ECMO-LP (leucine and pyruvate; n = 6), and ECMO-L (leucine only; n = 5). In five additional piglets, we also measured coronary flow and sampled coronary venous return for oxygen content to determine the ECMO effect on myocardial oxygen consumption. Coronary flow was determined in these experiments by means of a shunt placed in the coronary sinus as previously described (26, 27) with the hemiazygous vein ligated to prevent systemic venous flow contamination.

Metabolic Analyses

Preparation of myocardial extracts. Freeze-clamped sections of hearts in the region perfused by the left anterior descending coronary artery were pulverized under liquid nitrogen and the tissue extracted with either methanol/chloroform or 1.2 M perchloric acid, then neutralized with cold KOH to pH 7.4. The final supernatant was used for measurements in these experiments by means of a shunt placed in the coronary sinus as previously described (26, 27) with the hemiazygous

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GCMS (Fig. 3). Under these substrate provision conditions (leucine only), ECMO decreased the free leucine MPE by ~8% (LOAD-L vs. ECMO-L; $P < 0.05$).

Addition of isotopically labeled pyruvate decreased intracellular free leucine MPE by 15% in the LOAD groups (LOAD-L vs. LOAD-LP; $P < 0.005$). Pyruvate also decreased free leucine MPE in the piglets that underwent ECMO, but this did not reach significance (ECMO-L vs. ECMO-LP; $P = 0.08$). The ECMO-induced reduction in free leucine MPE noted for LOAD-L versus ECMO-L was not observed when pyruvate was included (LOAD-LP vs. ECMO-LP). Using $^{1}$H-NMR, we also analyzed absolute free amino acid concentration in LOAD-LP and ECMO-LP, and ECMO-L groups (Table 2). These data show that loading conditions did not influence free intracellular concentration for leucine and eight other amino acids, including the other branched chain amino acids valine and isoleucine. We compared ECMO-L and a separate ECMO group ($n = 5$) that did not receive leucine to determine whether our supraphysiological leucine doses altered free tissue leucine concentration. Leucine provided by coronary infusion did not alter tissue levels of leucine. Leucine infusion decreased tissue concentrations for the other branched chain amino acids, valine and isoleucine.

**Enrichment of CAC Intermediates**

Loading status alone did not significantly affect MPE of CAC intermediates determined by GCMS (for all intermediates, LOAD-L vs. ECMO-L and LOAD-LP vs. ECMO-LP; $P > 0.2$; Fig. 4). As expected, pyruvate increased CAC labeling by an average of 65% (LOAD-L vs. LOAD-LP, $P < 0.05$; ECMO-L vs. ECMO-LP, $P < 0.01$; Fig. 4). Loading status did not impact MPE of the pyruvate or lactate pools. As noted above, ECMO also did not affect enrichment of the free cytosolic leucine pool when pyruvate was provided. Thus the data imply that ECMO did not influence the uptake of pyruvate relative to leucine. This is subject to the assumption that sources of unlabeled pyruvate were not changed by ECMO. The systemic arterial levels of the primary alternate sources of pyruvate (glucose and lactate) were not altered by ECMO (data not shown), thereby supporting this assumption.

**Fractional Contributions of Acetyl-CoA to the CAC**

Figure 5A displays representative NMR complexes for glutamate. Our labeling strategy is such that the [2-$^{13}$C]-pyruvate labels C5 for glutamate, whereas [$^{13}$C$_6$,$^{15}$N]-L-leucine will label both C4 and C5 or only C4 allowing discrimination between the two (20). This labeling strategy also allows determination of the relative or fractional contributions via acetyl-CoA (Fc) to the CAC (Fig. 5B) by unlabeled endogenous substrates, labeled leucine, and labeled pyruvate by using the tcaCALC program. The loading state itself did not significantly change the $^{13}$C-leucine contribution (LOAD-L vs. ECMO-L, $P = 0.69$; LOAD-LP vs. ECMO-LP, $P = 0.26$) or the endogenous unlabeled substrate contribution (LOAD-L vs. ECMO-L, $P = 0.67$; LOAD-LP vs. ECMO-LP, $P = 0.67$). Similarly, loading status did not affect the $^{13}$C-pyruvate contribution (LOAD-LP vs. ECMO-LP; $P = 0.62$).

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**Table 1. Hemodynamic data**

<table>
<thead>
<tr>
<th></th>
<th>Starting</th>
<th>8 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LOAD</td>
<td>ECMO</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>98 ± 5</td>
<td>99 ± 4</td>
</tr>
<tr>
<td>Pressure, mmHg</td>
<td>8%</td>
<td>8%</td>
</tr>
<tr>
<td>Arterial</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>75 ± 3</td>
<td>78 ± 2</td>
</tr>
<tr>
<td>Diastolic</td>
<td>54 ± 2</td>
<td>56 ± 3</td>
</tr>
<tr>
<td>Mean</td>
<td>60 ± 2</td>
<td>63 ± 2</td>
</tr>
<tr>
<td>Pulse</td>
<td>21 ± 2</td>
<td>21 ± 2</td>
</tr>
<tr>
<td>Hemoglobin, g/dL</td>
<td>9.0 ± 0.6</td>
<td>9.2 ± 0.5</td>
</tr>
</tbody>
</table>

Values are means ± SE; $n = 11$ for all groups. $P$ values denote statistical comparisons between extracorporeal membrane oxygenation (ECMO) and piglets with normal circulation and not undergoing ECMO (LOAD) at each time point. The marked drop in pulse pressure demonstrates ventricular unloading by the ECMO circuit. NS, not significant.

Fig. 1. Myocardial oxygen consumption (MVO$_2$) during extracorporeal membrane oxygenation (ECMO; $n = 5$). MVO$_2$ (A) was determined through coronary sinus flow measurement (B) and systemic and coronary sinus blood sampling for O$_2$ content (C). ECMO decreases MVO$_2$ due to drop in A-V O$_2$ content difference. *$P < 0.05$ compared with pre (baseline before ECMO).
Under these conditions, hearts preferentially used pyruvate over leucine as an oxidative substrate, as expected. Pyruvate significantly decreased the FcLeucine in the LOAD condition by 67% (LOAD-L vs. LOAD-LP; \( P < 0.05 \)) and by 71% in ECMO (ECMO-L vs. ECMO-LP; \( P < 0.05 \)). Pyruvate also significantly decreased the unlabeled endogenous substrate contribution to glutamate via acetyl-CoA by 45% in LOAD (LOAD-L vs. LOAD-LP; \( P < 0.05 \)) and by 54% in ECMO (ECMO-L vs. ECMO-LP; \( P < 0.005 \)).

**Global Protein FSR**

The protein FSR was highest in the ECMO-L group (Fig. 6). ECMO increased the FSR by 25% compared with LOAD in piglets receiving leucine only, although this did not quite reach statistical significance (\( P = 0.06 \)). This effect was not observed in the presence of pyruvate (LOAD-LP vs. ECMO-LP; \( P = 0.92 \)). We infused leucine with targeted plasma levels, which were severalfold higher than physiological. Leucine stimulates protein synthesis in Langendorff hearts provided glucose as the sole alternative substrate and achieves maximal rates when provided at 3.7 mM (7–10). However, leucine at the same concentration does not elevate protein synthesis in working hearts when the heart is provided more robust and physiological substrates in the perfusate. We performed preliminary studies (\( n = 2 \)) with the same leucine intracoronary infusion, which showed substantially higher (near 2-fold) FSRs with reloading of the ventricles by weaning from ECMO (data not shown). Therefore, we are reasonably confident that we had not reached the maximal threshold for protein synthesis under our current conditions.

Although loading condition did not alter FSR, pyruvate did show a significant effect. Pyruvate reduced the FSR by 32% in ECMO (\( P < 0.01 \)) (ECMO-L vs. ECMO-LP). This same comparison did not reach significance in the LOAD groups (\( P = 0.31 \)).

**DISCUSSION**

The principal objective of our study was to identify ECMO promoted disturbances in amino acid shuttling between oxidation and protein synthesis in myocardium. ECMO provides relatively short-term recovery for the heart, presumably through ventricular unloading. We observed that unloading substantially reduces global myocardial oxidative metabolism, reflecting the decrease in cardiac work afforded by ECMO. ECMO also initiates an inflammatory cascade over the first few hours, which has been associated with end-organ insulin resistance. These hormonal abnormalities inhibit systemic carbohydrate utilization and enhances oxidation of alternate substrate sources (34). Whole body studies in infants have shown that ECMO promotes leucine oxidation, while increasing the protein degradation rate above synthesis rate and thereby leading to a deficit in protein balance and associated skeletal muscle wasting (34). It has been proposed that this branched chain amino acid undergoes catabolism instead of incorporation into protein and bypasses the insulin sensitive reactions to provide.

**Table 2. Free amino acid concentration in myocardial tissues by \(^1\)H-nuclear magnetic resonance spectrometry**

<table>
<thead>
<tr>
<th></th>
<th>LOAD</th>
<th>ECMO</th>
<th>No leucine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leucine</td>
<td>2.823 ± 294</td>
<td>3.225 ± 284</td>
<td>3.551 ± 209</td>
</tr>
<tr>
<td>Aspartate</td>
<td>1.009 ± 232</td>
<td>875 ± 220</td>
<td>1.115 ± 165</td>
</tr>
<tr>
<td>Glutamate</td>
<td>3.893 ± 325</td>
<td>3.808 ± 315</td>
<td>5.133 ± 386</td>
</tr>
<tr>
<td>Glutamine</td>
<td>7.324 ± 813</td>
<td>7.699 ± 1050</td>
<td>11.014 ± 1286</td>
</tr>
<tr>
<td>Glycine</td>
<td>1.801 ± 209</td>
<td>1.904 ± 172</td>
<td>1.637 ± 241</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>101 ± 21</td>
<td>134 ± 19</td>
<td>198 ± 31*</td>
</tr>
<tr>
<td>Leucine</td>
<td>181 ± 23</td>
<td>240 ± 30</td>
<td>278 ± 25</td>
</tr>
<tr>
<td>Threonine</td>
<td>533 ± 167</td>
<td>853 ± 84</td>
<td>908 ± 78</td>
</tr>
<tr>
<td>Valine</td>
<td>178 ± 29</td>
<td>247 ± 38</td>
<td>375 ± 57*</td>
</tr>
<tr>
<td>Glutamate/glutamine</td>
<td>0.55 ± 0.05</td>
<td>0.52 ± 0.06</td>
<td>0.50 ± 0.07</td>
</tr>
</tbody>
</table>

Values are means ± SE; \( n = 6 \) per group. *\( P < 0.05 \) vs. ECMO (leucine).
carbon substrate for oxidation within the CAC (34). Organ specificity for these metabolic disturbances has not been previously defined. Our results show that the heart resists some of these ECMO promoted metabolic perturbations. First, ECMO preserves myocardial leucine fractional contribution to CAC. As ECMO substantially reduces the myocardial oxygen consumption rate, and by extrapolation the total CAC flux, then the overall left ventricular leucine oxidation rate is reduced in contrast with the increase observed in whole body infant studies. Due to technical limitations in this small piglet model, we could not measure protein degradation rates. However, we demonstrated that ECMO maintains or marginally increases myocardial protein synthesis depending on the particular substrate provision. These results suggest that ECMO over this time period shifts these metabolic processes in heart toward more positive protein balance by preserving branched chain amino acids for protein synthesis rather than directing them into oxidation.

We used pyruvate both as a reference substrate for validation of fractional contribution to the CAC for leucine, and as an alternate substrate for glucose and other unlabeled substrates. We observed that pyruvate inhibited both leucine oxidation and fractional protein synthesis in piglets similarly under LOAD and ECMO. The directional changes are near proportionally equivalent for these two parameters. Therefore, they do not indicate a clear direction for protein balance. Consideration of results from other studies suggests that our observed rate changes generally accompany decreases in protein degradation as well as synthesis (34). Whole body studies in infants under ECMO showed that increasing caloric supply predominantly through glucose paradoxically exacerbates negative protein balance and increases leucine oxidation. Furthermore, high dose insulin infusion under euglycemic clamp and providing substantial amounts of glucose only marginally improves protein balance in infants under ECMO (2). Thus ECMO induced...
insulin resistance promotes whole body amino acid oxidation, which does not respond to high dose insulin. Although the experimental conditions and substrate provisions differ substantially between the human studies and our immature pig experiments, we have made certain poignant observations regarding substrate supply influence on metabolism, which specifically relate to the heart during ECMO. The heart supported by ECMO maintains metabolic flexibility similar to the LOAD condition by preferentially oxidizing pyruvate over unlabelled substrates. The unlabelled component is undefined and can include acetyl-CoA supplied by circulating glucose and fats, as well as glycogen and triglycerides within the heart. Pyruvate utilization appears to circumvent insulin resistance, and further preserve branched chain amino acids from oxidation. However, the inability of pyruvate to stimulate protein synthesis despite shifting leucine from amino acid oxidation illustrates the complexity of these relationships. Certainly further study using alternate substrates and labeling strategies will be necessary to explore regulation of protein turnover during ECMO.

We provided high target concentrations of isotopic leucine and pyruvate in these experiments. These concentrations were required to achieve the 13C-glutamate enrichment necessary for accurate NMR isotopic analyses. Although these concentrations are supraphysiological they provide reasonable caloric alternatives for immature hearts, considering that infants on ECMO are often supplied parenteral glucose at doses which yield hyperglycemia (2, 34). As leucine stimulates cardiomyocyte protein synthesis in vitro (36), elevating free cytosolic levels of this branched chain amino acid could conceivably influence and possibly maximize protein synthesis rates in our model in vivo. However, we provided evidence that the leucine loading into the coronary artery did not increase myocardial intracellular free leucine concentration in piglets undergoing ECMO. Consistent with prior studies (40), leucine infusion did decrease concentrations for both valine and isoleucine. Because concentrations for the other amino acids did not decrease, the data conform with previous contention that leucine promotes oxidative degradation of these other branched chain amino acids. The small decreases in the pool size of those branched chain amino acids might limit but certainly would not stimulate protein synthesis. Ichihara et al. (15) provided precedent for using supraphysiological leucine concentrations (1 and 5 mM) in coronary perfusate for isolated Langendorff and working hearts. The fractional contribution of acetyl-CoA from leucine under our conditions is small, but comparable with that reported in working rat hearts supplied leucine up to 5 mM (16%) (15). Previous studies have shown that 13C-labeled pyruvate generated from glucose maximally contributes ~60% of carbon within the CAC in heart, thereby illustrating a relative threshold for pyruvate dehydrogenase capacity (23).

Our GCMS results conform to this threshold range and are further confirmed by our NMR studies showing 13C-pyruvate fractional acetyl-CoA contribution to the CAC. With operation at this threshold in our experiments, pyruvate reduces the fractional acetyl-CoA contribution of leucine to the CAC in the current experiments. This finding is consistent with results from prior studies showing pyruvate (11 mM) inhibition of leucine oxidation in ex vivo perfused rat hearts (1).

The mechanism related to preferential oxidation of pyruvate over leucine has not been previously investigated in vivo. Pyruvate can potentially inhibit leucine metabolism at multiple steps along the pathway beginning at plasma membrane and/or mitochondrial transport and extending through catabolism to acetoacetate and acetyl-CoA. The observed manipulation of fractional leucine contribution of acetyl-CoA by pyruvate could relate to trans-sarcolemmal transport, since pyruvate lowers isotopic enrichment of free intracellular leucine pool significantly in the LOAD group, although just marginally in the ECMO hearts. Potentially, an increase in protein degradation could release more unlabeled leucine into cytoplasm, thereby diluting the isotopic enriched pool, and providing an alternative interpretation for this data. As noted, whole body studies using similar 13C-leucine studies during ECMO have shown that an increase in leucine oxidation accompanies elevated rates of protein degradation (2). Instead, we found that pyruvate decreases the relative acetyl-CoA contribution from leucine. Therefore, we assumed that leucine release from protein degradation is negligible with respect to our calculations. Within these limitations, the data imply that pyruvate modification of leucine transport is responsible for at least a portion of the decrement in FcLeucine. Leucine transport into the cell is regulated by multiple amino acid transporters, predominantly by the sodium-independent system-L amino acid transporter-1. Although exercise upregulates the sodium-independent system-L amino acid transporter-1 in skeletal muscle, prior studies have suggested that myocardial transport likely depends more on undefined posttranslational modification of these amino acid transporters (12).

With the consideration that there was only a small decrement in free cytosolic leucine enrichment, it is unlikely that alteration in transport as discussed above is totally responsible for the marked pyruvate effect on leucine oxidation. Pyruvate inhibition of leucine oxidation in heart has been previously linked to reversible modifications in the branch-chain α-keto-dehydrogenase (BCKD) (38). This enzyme complex resides in the mitochondria and catalyzes oxidative decarboxylation of the α-isoketocaprate, the product of reversible leucine transamination (4–6). Pyruvate provided in low doses (0.25 mM) to isolated Langendorff perfused hearts markedly inhibited branch-chain α-keto-dehydrogenase activity with no further increase in response up to 10 mM (38). Results in our study are consistent with a mode of posttranslational modification of this enzyme, which has yet to be fully described. These same modifications could explain the ECMO promoted shift from leucine oxidation to protein incorporation.

**Limitations and Experimental Considerations**

Our experiments evaluated cardiac leucine oxidation and protein synthesis using ECMO, a very specific form of mechanical circulatory unloading commonly used in infants and children. We found that metabolic flexibility was maintained during ECMO. Thus we did not directly link a metabolic impairment with cardiac stunning, which often occurs during this critical time period in human infants. However, we established baseline parameters for our ECMO model, which are necessary for further study and exploration of those potential links.

Difficulties exist when trying to extrapolate our findings to other forms of therapeutic ventricular unloading. For example, ventricular assist devices (VAD) use markedly different circu-
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Latory strategies, are intended for much longer periods of support, and are generally used in older populations with chronic maladaptive left ventricular hypertrophy. Although signaling for these processes has been examined in experimental models of unloading (11, 29, 30) and in adults (37) and children with ventricular assist devices (24), no prior study has actually measured their influence on protein synthesis or amino acid oxidation. We attempted to evaluate signaling for protein synthesis in this pig model using standard immunoblot techniques. However, inconsistent antibody performance in pig myocardium as well as inherent variability for this species limited our ability to attain definitive results.

Ventricular remodeling associated with chronic unloading produces alterations in expression of specific proteins. Our objectives were to study the integration between myocardial amino acid oxidation and protein synthesis, which was relevant to short-term ECMO. Therefore, we did not attempt to perform technically difficult measures of FSRs for specific proteins. These specific types of analyses are planned for the future.

Protein turnover depends on rates of both synthesis and degradation. Our experimental design did not include the technologically difficult measurements of myocardial protein degradation. Therefore, we cannot comment on total protein turnover but instead focused on integration between amino acid degradation and CAC metabolism.

Finally, we studied metabolism after an 8-h period of ECMO. This time period is critical for pediatric patients undergoing these procedures for cardiac indications. This duration also represents the previous standard for most experimental ECMO studies in pigs, due to stability and logistical concerns. Additionally, we showed that by 8 h the inflammatory response is beginning to subside. However, the translation to the clinical scenario is limited as most patients undergo ECMO for somewhat longer time periods.

In summary, we found that ECMO decreases leucine oxidation, while preserving protein synthesis and metabolic flexibility for substrate oxidation. Pyruvate provides an alternate carbon source, which reduces protein synthesis but also decreases leucine oxidation, and possibly improving net protein balance.

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Disclosures

No conflicts of interest, financial or otherwise, are declared by the author(s).

Author Contributions


References


