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Ventricle-Specific Metabolic Differences in the Newborn Piglet Myocardium *In Vivo* and During Arrested Global Ischemia

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ABSTRACT: Ventricular dysfunction is reported greater in the left (LV) *versus* right ventricle (RV) in infants following surgically induced ischemia. Ventricle-specific differences in baseline metabolism may alter response to ischemia thus affecting postischemic functional recovery. This study identifies ventricle-specific metabolic differences in the newborn (piglet) heart at baseline (working) and during ischemia (arrested). Baseline LV citrate synthase (CS) and hydroxyacyl-CoA dehydrogenase (HAD) activities were 15% and 18% lower ($p < 0.02$), whereas creatine kinase (CK) and phosphofructokinase (PFK) activities were 40% and 23% higher ($p < 0.04$) than the RV. Baseline LV glycogen reserves were also 55% higher ($p = 0.004$). By 15 min of ischemia, LV ATP was 20% lower ($p < 0.05$), lactate was 51% higher ($p = 0.001$), and hydrogen ions (H^+) were 43% higher ($p = 0.03$) compared with the RV. These differences persisted for the entire ischemic period ($p < 0.02$). After 45 min of ischemia, the LV used 58% less ($p < 0.05$) glycogen than the RV. These findings demonstrate that the enhanced glycolytic capacity of the newborn LV was accompanied by greater anaerobic end-product accumulation and lower energy levels during ischemia. This profile may offer one explanation for greater LV-dysfunction relative to the RV in children following ischemia. **(*Pediatr Res* 63: 15–19, 2008)**

Clinical studies continue to report persistent postoperative dysfunction in the left ventricle (LV) of children even after successful repair of pathology affecting the right ventricle (RV) (1,2). Experimental studies in the newborn heart suggest a lower tolerance of the LV to ischemia, as LV recovery of function following ischemia is far worse than that seen in the RV (3). Whether these findings are a result of ventricle-specific differences in postnatal myocardial metabolic maturation is currently unknown.

During postnatal cardiac maturation, the LV undergoes rapid "physiologic" hypertrophy relative to the RV in response to postnatal changes in systemic and pulmonary circulation (4). Morphologic studies using newborn animals have reported a lower capillary density in the LV relative to the RV as noted by greater LV myocyte-to-capillary ratios and intercapillary distances (5). Lower tissue perfusion capacity in the face of increased postnatal ventricular workloads and rapid ventricular growth in the newborn LV may result in underperfusion. Whether this has detrimental effects on metabolic capacity and response to ischemia is unknown.

During postnatal cardiac maturation, there is a shift in energy substrate reliance where the use of fatty acids dramatically increases and that of glucose decreases (6–9). Central to this transition is an early decrease in the activity of key enzymes involved in the glycolytic pathway and an increase in the enzymes responsible for fatty acid and oxidative metabolism (8,9). Interestingly, studies have shown that adult hearts adapt to chronic increases in ventricular workloads by shifting energy substrate reliance away from fatty acid and oxidative pathways back to glucose and glycolytic processes, thus taking on a more "fetal-like" metabolic profile (10–12). The question arises as to whether the normal postnatal shift from carbohydrate to fatty acid and oxidative metabolism (6–9) is delayed in the rapidly growing newborn LV relative to the RV. This is especially pertinent in the face of a perfusion mismatch in the newborn heart.

Studies have shown that hearts more reliant on carbohydrate metabolism have an enhanced glycolytic capacity, which results in the more rapid accumulation of lactate and hydrogen ions (H^+) during ischemia (13–15). These anaerobic end products can subsequently lead to premature inhibition of glycolysis (14–16), more rapid depletion of adenosine triphosphate (ATP), decreased myocardial ischemic tolerance (14,15), and decreased postischemic functional recovery (13,17,18). Ventricle-specific differences in such metabolic responses to ischemia may therefore influence the susceptibility of a child's heart to injury when undergoing surgically induced ischemia, and offer a potential explanation for studies that have reported greater LV dysfunction following cardiac surgery.

The purpose of this study was thus to investigate whether ventricle-specific metabolic adaptations exist in the newborn working heart at baseline, and whether these adaptations affect the heart's metabolic response to global ischemia under arrested conditions.

MATERIALS AND METHODS

Protocol. All experimental procedures were approved by the University of Toronto Animal Care and Use Committee, and follow the rules set out by the

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Abbreviations: CK, creatine kinase (EC 2.7.3.2); **CP,** creatine phosphate; **CS,** citrate synthase (EC 4.1.3.7); **H^+,** hydrogen ion; **HAD,** hydroxyacyl-CoA dehydrogenase (EC 1.1.1.35); **LDH,** lactate dehydrogenase (EC 1.1.1.27); **LV,** left ventricle; **PFK,** phosphofructokinase (EC 2.7.1.11); **RV,** right ventricle

Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (National Institutes of Health publication No 85-23, revised 1996). Newborn (3-d-old) Yorkshire piglets ($n = 7$) were anesthetized with Somnotol (sodium pentobarbital 65 mg/mL, MTC Pharmaceuticals, Cambridge, ON, Canada), intubated, and mechanically ventilated to normoxia. The right carotid artery was catheterized to ensure normal blood gases and pH. Following a midline sternotomy, the working heart was exposed and full thickness LV and RV *in vivo* biopsies were taken using a freeze-clamp technique (19). The heart was then excised, marking the onset of global myocardial ischemia, and placed in glucose-free Krebs-Henseleit physiologic solution at 38°C. Myocardial contractions ceased immediately following organ excision. Serial full-thickness LV and RV freeze clamp biopsies were taken from the arrested heart at 15, 30, and 45 min of ischemia. All biopsies were stored at −85°C until biochemical analysis.

Biochemistry. To determine possible ventricle-specific differences in metabolic potential, the maximum activities of key enzymes involved in fatty acid and carbohydrate, oxidative and nonoxidative metabolism were measured in baseline tissue using fluorometric assays (20). The following enzyme activities (mmol/g protein/h) were measured: hydroxyaceyl-CoA dehydrogenase (HAD – fatty acid breakdown), phosphofructokinase (PFK- glycolysis), citrate synthase (CS – Kreb's cycle), lactate dehydrogenase (LDH – anaerobic glycolysis), and creatine kinase (CK – creatine phosphate (CP) shuttle). A PFK/CS ratio was also calculated as it reflects a tissue's relative reliance on carbohydrate *versus* oxidative metabolism. Ventricular baseline and ischemic metabolism was characterized through the measurement of tissue energy levels (ATP and CP), anaerobic substrate reserves (glycogen), and metabolic end products (lactate and H^+). Biochemical analysis for ATP, CP, glycogen, and lactate (μmol/g dry weight) involved using fluorometric assays (21) whereas H^+ content (mol/L) was determined using the homogenate method (22).

Data analysis. All values are expressed as mean ± SD. *In vivo* LV and RV differences were compared using a paired *t* test. A two-way repeated measures ANOVA with Bonferroni post hoc test was used to compare differences within a ventricle and between ventricles overtime during ischemia. Significance was defined as $p < 0.05$.

Table 1. *Maximum enzyme activities in the RV and LV*

Enzyme	RV	LV
CK	72.3 ± 23.3	99.1 ± 25.8*
PFK	7.4 ± 1.9	9.1 ± 2.4*
CS	16.7 ± 2.5	14.2 ± 1.7*
HAD	16.1 ± 4.2	13.2 ± 4.6*
LDH	51.3 ± 12.5	48.4 ± 6.6
PFK/CS	0.44 ± 0.07	0.64 ± 0.15*

LV and RV enzyme activity (mmol/g protein/h). Values expressed as mean ± SD.

* $p < 0.03$.

RESULTS

Metabolic enzyme activities. Relative to the RV, activities of enzymes involved in fatty acid (HAD) and oxidative metabolism (CS) were significantly lower ($p < 0.02$) in the LV (Table 1). In contrast, PFK activity was significantly higher in the LV ($p = 0.041$), as was the PFK/CS ratio ($p = 0.001$). CK activity was also significantly higher ($p = 0.001$) in the LV relative to the RV. Other enzymes measured showed no statistical differences.

ATP. *In vivo* ATP values were similar in the LV and RV (Fig. 1) and reflect values from previous reports in the newborn piglet (23). After 15 min of global ischemia, ATP levels declined significantly in both ventricles, however, LV ATP was 20% lower ($p = 0.04$) than the RV. This ventricular difference persisted ($p < 0.02$) until the end of the ischemic period, with LV ATP being 30% lower than RV ATP at 45 min of ischemia.

CP. LV *in vivo* CP levels were similar to RV levels (Fig. 2) and reflect values from previous reports in the newborn piglet (23). After 15 min of ischemia, CP reserves were almost completely depleted in both ventricles.

Glycogen. *In vivo* LV glycogen reserves were significantly higher ($p = 0.004$) than the RV (Fig. 3). Both values for the LV and RV fall within the range reported previously but separately in the literature (16,24). After 15 min of ischemia, there was a statistically significant 15% LV and 22% RV reduction ($p < 0.02$) in glycogen levels. No further significant reductions in glycogen levels occurred in the LV during the rest of ischemia, while in the RV glycogen levels continued to decrease by an additional significant 37% ($p = 0.003$) after 45 min. Thus, compared with the RV, LV glycogen reserves remained significantly higher throughout the entire ischemic period ($p < 0.001$). At 45 min of ischemia, glycogen use was incomplete in both ventricles, with the LV utilizing only 23% of its glycogen reserves, whereas the RV used 57%.

Lactate. *In vivo* lactate levels were comparable in each ventricle (Fig. 4*A*) and reflect values within the physiologic range for the newborn perfused heart (23). By 15 min of global ischemia, lactate accumulated significantly ($p < 0.001$) in both ventricles but the LV accumulated 51% more lactate

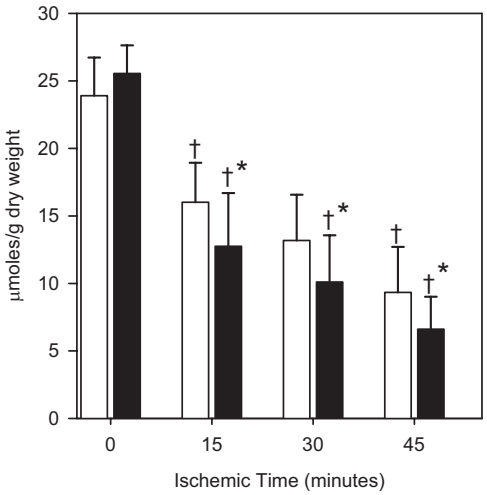

Figure 1. ATP levels *in vivo* and at 15, 30, and 45 min of ischemia in newborn piglet LV and RV. Values expressed as mean ± SD. †$p < 0.02$ *vs* previous time within a ventricle, and *$p < 0.04$ *vs* RV at same time point. RV (*open bar*); LV (*closed bar*).

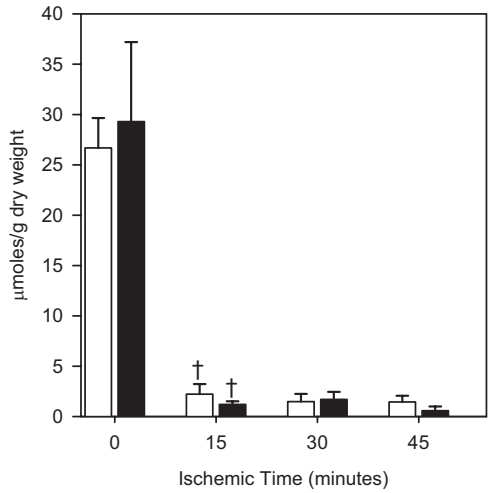

Figure 2. CP levels *in vivo* and at 15, 30, and 45 min of ischemia in newborn piglet LV and RV. Values expressed as mean ± SD. †$p < 0.001$ *vs* previous time within a ventricle. RV (*open bar*); LV (*closed bar*).

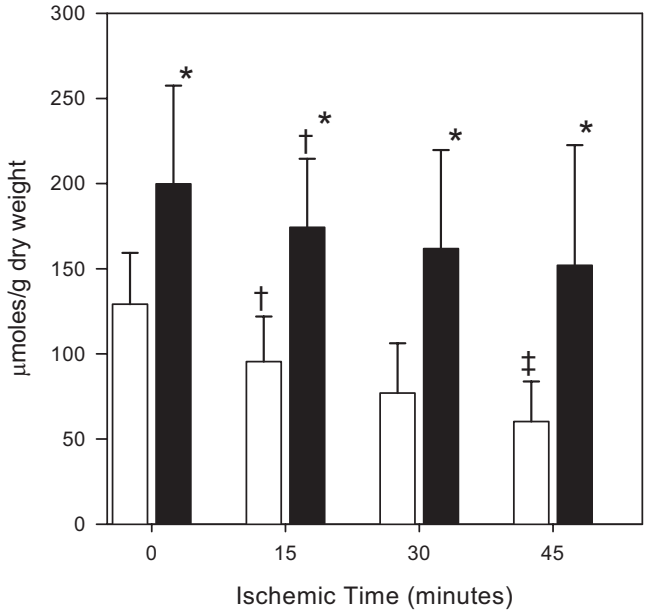

Figure 3. Glycogen levels *in vivo* and at 15, 30, and 45 min of ischemia in newborn piglet LV and RV. Values expressed as mean ± SD. †$p < 0.02$ *vs* control within a ventricle, ‡$p < 0.02$ *vs* RV at 15 min, and *$p < 0.002$ *vs* RV at same time point. RV (*open bar*); LV (*closed bar*).

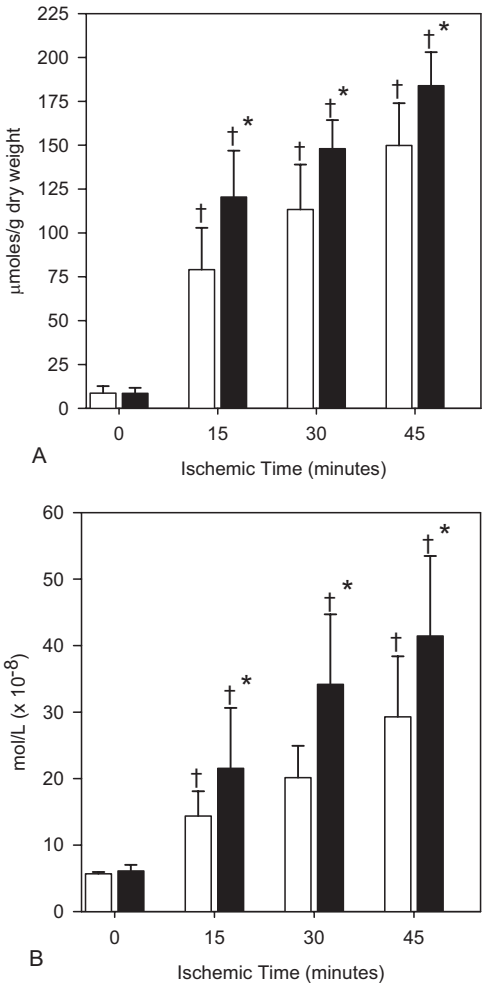

Figure 4. (*A*) Lactate and (*B*) H^+ levels *in vivo* and at 15, 30, and 45 min of ischemia in newborn piglet LV and RV. Values expressed as mean ± SD. †$p < 0.004$ *vs* previous time within a ventricle, and *$p < 0.03$ *vs* RV at same time point. RV (*open bar*); LV (*closed bar*).

compared with the RV ($p < 0.001$). With further ischemia, both ventricles continued to significantly accumulate ($p < 0.001$) lactate, however, the LV always exhibited 20–25% higher levels of lactate ($p < 0.001$).

H^+. There were no significant *in vivo* differences between LV and RV H^+ levels (Fig. 4*B*), which also fell within the physiologic range for newborn hearts (25). After 15 min of ischemia, although H^+ content significantly increased in the both ventricles ($p < 0.001$), the LV exhibited a significant 43% higher ($p = 0.03$) H^+ content compared with the RV. This difference persisted throughout the ischemic period ($p < 0.002$).

DISCUSSION

This work identified for the first time that the LV and RV of the newborn heart are not metabolically identical and that they responded differently to global myocardial ischemia. The LV of the newborn heart demonstrated an enhanced glycolytic capacity, which during ischemia was associated with greater anaerobic end-product accumulation and lower energy levels. These novel ventricle-specific differences expand our understanding of postnatal myocardial maturation as anatomical, histologic, and functional differences between the LV and RV in newborns now include metabolic parameters.

Baseline enzyme potential. Experimental studies in adults and clinical reports in children have reported that chronic increases in ventricular workloads cause a shift in the potential for energy metabolism away from fatty acid and oxidative pathways and back to carbohydrate and glycolytic processes (10–12,26). Similarly, perinatal changes in systemic/pulmonary circulation and hemodynamics results in dramatic increases in LV workloads relative to the RV (4), which may act to prolong a more "fetal-like" metabolic profile in the LV. Interestingly, in this study, the newborn LV demonstrated a greater potential for carbohydrate *versus* fatty acid and oxidative metabolism compared with the RV. This was demonstrated by higher PFK activity and lower HAD and CS activity in the LV compared with the RV. These ventricle-specific differences may also in part arise from possible differences in tissue perfusion. Studies in newborn lambs have reported a transient postnatal period in which the LV has significantly lower capillary density, greater intercapillary distance and increased myocyte-to-capillary ratios compared with the RV (5). A greater glycolytic potential may therefore be an important adaptive mechanism of the LV as glucose metabolism provides more ATP per mole O_2 compared with fatty acid metabolism.

The importance of a possible tissue perfusion mismatch in determining ventricle-specific differences in metabolic potential is furthermore highlighted by studies using smaller animal newborn models. For example, in studies using newborn rats and guinea pigs in which a perfusion mismatch has not been reported in the LV (27), only minimal ventricle-specific differences in enzyme activity have been identified (8,9). These results suggest that ventricle-specific differences in enzyme capacity may be a phenomenon present solely in larger more

human-like animal models in which a possible perfusion mismatch has been reported in the newborn LV.

Perinatal changes in ventricular workloads and tissue perfusion may also confer a greater anaerobic *versus* aerobic enzyme potential in the LV. Although no differences in LDH activity was noted in this study, the actual activity of LDH was 5–7 times greater than the activity of PFK. This suggests that PFK and not LDH may act as the rate-limiting step in anaerobic glycolysis. Higher PFK activity in the LV relative to the RV therefore confers a greater anaerobic potential to the LV in the newborn heart. A higher PFK/CS ratio in the LV compared with the RV further demonstrates this greater anaerobic *versus* aerobic metabolic potential of the LV. These enzyme profiles confirm the LV's more "fetal-like" metabolic potential.

It is interesting to note that the only enzyme that did not display a more "fetal-like" profile in the newborn LV was CK. CK is the enzyme responsible for the reversible transfer of high-energy phosphates between creatine and ATP, and strongly reflects the ability of the myocardium to increase its contractile performance (28). At birth, CK is relatively immature and its activity increases during postnatal maturation (29). In this study, CK activity was significantly greater in the LV relative to the RV, indicating that a more mature CK profile may be needed to support its greater workloads and more rapid growth. Furthermore, because CK isoforms are coupled to both sites of ATP production (mitochondria) and ATP consumption (myofibers and cell membranes), a higher total CK activity may therefore help to maintain normal cellular bioenergetics (ATP and CP) in the more energy demanding LV.

Baseline metabolites. A greater reliance on carbohydrate metabolism may be more efficient based on O_2 consumption. However, it has considerably less ATP yield per carbon of substrate relative to use of fatty acids. This may significantly limit the ATP producing power of the LV during postnatal development. The results of this study, however, suggest that the newborn LV may compensate for this by maintaining higher stores of myocardial glycogen. Glucose derived from glycogen not only provides a greater net ATP yield *via* glycolysis, but it is preferentially oxidized compared with exogenous glucose. This ensures the highest possible ATP yield from carbohydrate substrates (12,30). These results are also in line with the hypothesis that the LV has a more "fetal-like" metabolic profile relative to the RV, as developmentally, myocardial glycogen levels are reported to rapidly decline following birth (31).

Response during ischemia. Studies comparing newborn and adult hearts have long since recognized the enhanced glycolytic capacity of the newborn myocardium (14–17). Newborn hearts during ischemia are reported to have a more rapid depletion of high-energy phosphates and greater accumulation of anaerobic end products (14–16). Glycolytic enzymes, such as PFK have been shown to be inhibited by anaerobic end products (14,15). The more rapid and greater development of lactic acidosis during ischemia may result in the reduction or complete inhibition of glycolysis, thereby exacerbating ATP depletion. In this study, it is therefore not unreasonable to suggest that the greater accumulation of anaerobic end products demonstrated in the newborn LV may ultimately reduce its glycolytic rate resulting in more rapid ATP depletion compared with the RV.

During global myocardial ischemia, myocardial glycogen becomes one of the key energy substrates available for ATP production. In adults, higher myocardial glycogen reserves are believed to be cardioprotective during a hypoxic stress, as it may prolong energy generation *via* anaerobic glycolysis (32). Alternatively, during an ischemic stress in adults, higher glycogen reserves have been associated with a greater accumulation of glycolytic end products and decreased postischemic ventricular function (17). In this study, the ventricle with higher glycogen (*i.e.* LV) also accumulated more lactate and H^+ during ischemia. Interestingly, both ventricles also failed to completely use their glycogen reserves in the face of rapid energy depletion during ischemia. This effect was exacerbated in the LV. Previous studies have suggested that incomplete glycogen utilization in the newborn heart results from anaerobic end-product feedback inhibition of glycolysis which was confirmed by significant buildup of glucose-6-phosphate levels (15,16). Interestingly, in a small subset of piglets in which 5-min ischemic biopsies were assayed for glycogen, it was found that the LV broke down 6 times more glycogen than the RV. This more rapid rate of early glycogenolysis in the newborn LV may flood the cell with glucose-6-phosphate resulting in cessation of further glycogenolysis with continued ischemia. These results therefore also suggest ventricle-specific differences in the control of glycogenolysis in the newborn heart.

One of the potential limitations of this study is the lack of a direct correlation between enzyme activity and the rate of glycolytic and oxidative metabolism. The *in vitro* assay used in this study measures the maximum potential rate of each enzyme-catalyzed reaction in an optimum environment free of allosteric modifiers. This activity, however, may differ *in vivo* as enzyme activity is constantly being modified by changing concentrations of substrates, products and modifiers. Despite this limitation, other studies using a similar method to measure enzyme activity have associated greater enzyme activity with increased rates of substrate utilization (11) further validating this measurement.

Compared with the RV, the metabolic response of the newborn LV may place it at greater metabolic risk during ischemia. Studies have identified that more rapid depletion of high-energy phosphates in the newborn heart is associated with a more rapid onset of ischemic contracture and increased postischemic ventricular dysfunction (15,24,33). In human infants undergoing cardiac surgery, a rather small difference in ischemic ATP levels (4 μmol/g dry weight) was correlated with worse postischemic function, longer time spent in the ICU, and longer hospital stays (33). Other investigators have suggested that accumulation of anaerobic end products and not reduced ATP levels is responsible for damage to the myocardium during ischemia (17). H^+ accumulation is believed to trigger an ion exchange sequence ultimately resulting in mitochondrial damage, cell death, and significant myocardial dysfunction following reperfusion (16,17,34). In this study, the LV of each animal consistently had lower ATP and higher anaerobic end products during ischemia when compared with its RV. These findings suggest that the newborn LV is at greater metabolic risk during ischemia. Whether these

differences also translate into functional differences following reperfusion and resumption of workload, as reported in other studies, remains to be confirmed.

In the clinical setting, despite the utilization of cardioprotective techniques, such as hypothermia and cardioplegic solutions, studies have still reported greater left ventricular postischemic dysfunction in children following repair of RV pathology (1,2). There is also currently no evidence to suggest that cardioplegic solutions offer a similar level of protection for both the LV and RV in the pediatric population. The employment of Kreb's physiologic solution in this study, allowed for the comparison of left and right ventricular responses without the additional confounding affects of cardioplegia. Should these differences between the LV and RV hold in the clinical setting, this may offer a potential explanation for studies that have reported greater left ventricular postischemic dysfunction in normal newborn hearts and newborn hearts with right-sided pathology (1–3). In addition, caution should be exercised by both experimental and clinical investigators using RV biopsies to assess LV metabolism and function in the pediatric population (33,35), as they may be underestimating the potential risk factors associated with ischemia.

Ventricle-specific differences in the susceptibility to ischemia may be more than a newborn phenomenon and continue with postnatal development. Pediatric studies that have reported LV dysfunction following repair of RV pathology include children well outside of the newborn age (1). Interestingly, studies in healthy adult hearts have already failed to identify ventricle-specific differences in enzyme activity (36) and metabolite levels at baseline and during ischemia (36,37). These results therefore suggest that ventricle-specific metabolic differences may exist outside of the newborn age but remain specific to young children with a cardiac pathology.

This work presents the novel findings that within a nonpathological newborn heart the LV and RV show dramatic metabolic differences in baseline metabolism and their response to ischemia. Relative to the RV, the newborn LV demonstrates a more "fetal-like" metabolic profile such that it may have a greater preference for carbohydrate *versus* fatty acid metabolism and greater enzyme potential for anaerobic *versus* aerobic metabolism. The enhanced glycolytic capacity of the LV during ischemia results in a greater accumulation of anaerobic end products and reduced ischemic energy levels potentially placing the LV at greater risk of ischemic injury. Using this new information, greater emphasis on designing ventricle-specific cardioprotective strategies may be required to account for the metabolic differences of the LV and RV and to reduce possible ischemic injury anytime there is stress or restricted oxygen delivery to the newborn heart.

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