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Sex Differences in Newborn Myocardial Metabolism and Response to Ischemia

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ABSTRACT: In children with congenital heart disease, female sex has been linked to greater in-hospital mortality associated with low cardiac output, yet the reasons for this are unclear. Therefore, we examined whether newborn sex differences in the heart's metabolic response to ischemia exist. Left ventricular (LV) *in vivo* and ischemic biopsies of newborn male and female piglets were compared. Tissue ATP, creatine phosphate (CP), glycogen, anaerobic end-products lactate and hydrogen ion (H^+), and key regulatory enzymes were measured. Compared with males, newborn females displayed 14% lower ATP, 22% lower CP, and 32% lower glycogen reserves ($p < 0.05$) at baseline. During ischemia, newborn females accumulated 17% greater lactate and 40% greater H^+ accumulation ($p < 0.02$), which was associated with earlier cessation of glycolysis and lower ischemic ATP levels ($p < 0.02$) compared with males. Newborn females demonstrated a greater ability to use their glycogen reserves, resulting in significantly lower ($p < 0.003$) glycogen levels throughout the ischemic period. Thus, newborn females are at a metabolic disadvantage because they exhibited lower energy levels and greater tissue lactic acidosis, both linked to an increase susceptibility to ischemic injury and impair myocardial function on reperfusion. **(*Pediatr Res* 70: 148–152, 2011)**

Several studies in children have documented worse outcome of females relative to males during management or after repair for congenital heart disease (1–4). Chang et al. (2) reported that female sex was associated with 51% higher odds of mortality after cardiac surgery with the highest odds of mortality being in newborns. A 2007 nationwide study confirmed this finding and reported a 31% greater risk of mortality in female children during hospitalization for cardiac surgery (4). Compared with males, other studies have also reported lower survival of female children requiring mechanical cardiopulmonary support for severe postoperative ventricular dysfunction (1,3). Although these findings suggest that even in children, female sex may influence outcome following periods of myocardial ischemia or oxygen stress, the metabolic explanation by which this may occur is currently unknown.

In the adult heart, experimental studies have already reported sex-specific differences in myocardial metabolism and response to ischemia that may potentially impair ischemic tolerance and reduce postischemic myocardial functional recovery. Compared with males, the adult female myocardium is believed to have a greater reliance on carbohydrate and glycolytic metabolism, which during ischemia is associated with a more rapid accumulation of anaerobic end-products (5–7) and increased postischemic ventricular dysfunction (6,8). In the newborn heart, experimental studies have also associated a more rapid accumulation of anaerobic end-products with premature inhibition of glycolysis (9–11), more rapid depletion of ATP, decreased myocardial ischemic tolerance (9,10), and lower postischemic functional recovery (12,13). Because in the pediatric population, low cardiac output following myocardial ischemia has been reported to be the most common cause of morbidity and mortality after repair of congenital lesions (14,15), sex-specific differences in anaerobic end-product accumulation and/or cellular energy levels during ischemia may offer a potential explanation for differences in operative outcome in male *versus* female children. The purpose of this study was therefore to identify sex-specific differences in baseline and ischemic metabolism in the newborn myocardium and to explore the possible metabolic explanation for this.

MATERIALS AND METHODS

All experimental procedures were approved by the University of Toronto Animal Care and Use Committee and follow the rules set out by the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (National Institutes of Health publication No. 85-23, revised 1996).

Neonatal Yorkshire piglets were used in this study because the degree of maturity of the cardiovascular system is structurally and functionally similar to that of newborn humans (16,17). In addition, their plasma hormone levels have been reported as low to nondetectible at this age (18). Male ($n = 7$) and female ($n = 13$) age-matched newborn (3 d old) piglets (male: 2.0 ± 0.07 kg *versus* female: 2.0 ± 0.11 kg, $p = 0.883$) were anesthetized with Somnotol (Sodium Pentobarbital 65 mg/mL; MTC Pharmaceuticals, Cambridge, Canada), intubated, and mechanically ventilated. An indwelling cannula was placed in the right carotid artery to monitor blood gases and blood pressure. Appropriate adjustments were made to ventilation to ensure normal blood gas and pH (Table 1). After a midline sternotomy, the heart was exposed, and a full-thickness freeze-clamp left ventricular (LV) biopsy was taken (19). Immediately after the *in vivo* biopsies, the heart was excised marking the onset of global myocardial ischemia and placed in substrate-free Krebs-Henseleit physiologic solution at 38°C during which serial LV biopsies were taken at 30, 45, and 60 min of ischemia. Previous work with this animal model demonstrated that at 30 min of ischemia, moderate impairments of myocardial function are noted, whereas 45 min or longer of ischemia resulted in profound

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Abbreviations: CK, creatine kinase; **CP,** creatine phosphate; **GP,** glycogen phosphorylase; **HK,** hexokinase; **LDH,** lactate dehydrogenase; **LV,** left ventricular; **PFK,** phosphofructokinase

Table 1. *Hemodynamics and blood gas parameters*

	Males	Females
Hemodynamics		
HR (bpm)	139 ± 11	169 ± 6*
SBP (mm Hg)	70 ± 4	75 ± 2
DBP (mm Hg)	35 ± 3	39 ± 2
MAP (mm Hg)	47 ± 3	51 ± 2
Blood gas and electrolytes		
pH	7.40 ± 0.01	7.39 ± 0.01
pO_2 (mmol/L)	100 ± 6	96 ± 3
$satO_2$ (%)	102 ± 0.4	101 ± 0.6
pCO_2 (mmol/L)	39.3 ± 1.8	38.5 ± 1.2
HCO_3^- (mmol/L)	24.0 ± 1.0	23.6 ± 0.4
BE	−0.10 ± 0.9	−0.9 ± 0.5
Glucose (mmol/L)	4.4 ± 0.5	4.5 ± 0.2
Lactate (mmol/L)	3.1 ± 0.6	2.5 ± 0.3
Hematocrit (%)	29.0 ± 1.8	30.5 ± 1.4

Newborn male and female hemodynamics and blood gas parameters. Values expressed as mean ± SEM.

* $p < 0.03$ *vs* males.

HR, heart rate; bpm, beats per minute; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; BE, base excess; $satO_2$, oxygen saturation.

functional impairment (13). All biopsies were stored at −85°C until biochemical analysis.

To determine possible sex-specific differences in metabolic potential, the maximum activities of key enzymes involved in the creatine phosphate (CP) shuttle and anaerobic glycolysis were measured using fluorometric assays (20). The following enzyme activities (mmoles/g protein/h) were measured: creatine kinase (CK, CP shuttle), glycogen phosphorylase (GP, glycogenolysis), hexokinase (HK, glucose phosphorylation), phosphofructokinase (PFK, glycolysis), and lactate dehydrogenase (LDH, anaerobic glycolysis). Sex-specific differences in myocardial baseline and ischemic metabolism were characterized through the measurement of tissue energy levels (ATP and CP), anaerobic substrate reserves (glycogen), and metabolic end-products [lactate and hydrogen ions (H^+)]. Biochemical analysis for ATP, CP, glycogen, and lactate (μmoles/g dry weight) involved using fluorometric assays (21), whereas H^+ content (mol/L) was determined using the homogenate method (22,23).

All values are expressed as mean ± SEM. *In vivo* male and female data were compared using *t* tests (two-tailed), whereas a two-way repeated measures ANOVA with Bonferroni *post hoc* test was used to compare differences between sexes over time. Significance was defined as $p < 0.05$.

RESULTS

Hemodynamics and blood gas parameters. Male and female hemodynamics and blood gas parameters are presented in Table 1. Systolic, diastolic, and mean arterial pressures were similar between males and female newborn piglets. Females, however, demonstrated a significant 22% higher resting heart rate compared with males. Blood gas, pH, electrolytes, and hematocrit were similar between newborn male and female piglets. All hemodynamic and blood gas parameters fall within the normal range reported in the literature for male piglets (24).

ATP. Newborn male and female *in vivo* and ischemic ATP levels are presented in Figure 1. Newborn female *in vivo* myocardial ATP levels were a significant 14% lower compared with males (males: 25.5 ± 0.9; females: 22.0 ± 0.7; $p = 0.005$). Overall, during ischemia, there was a significant decline in myocardial ATP levels ($p < 0.03$) at each ischemic time interval in both sexes. Despite females having lower levels at baseline, at 30 min of ischemia both sexes reached a similar level of ATP ($p = 0.66$). With continued ischemia,

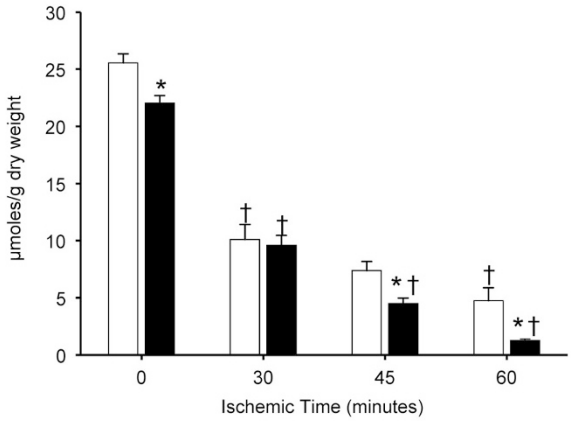

Figure 1. ATP levels *in vivo* and at 30, 45, and 60 min of ischemia in newborn male and female piglets. Values expressed as mean ± SEM: *$p < 0.02$ *vs* opposite gender at same time point, †$p < 0.003$ *vs* previous time within a gender. Males (□); females (■).

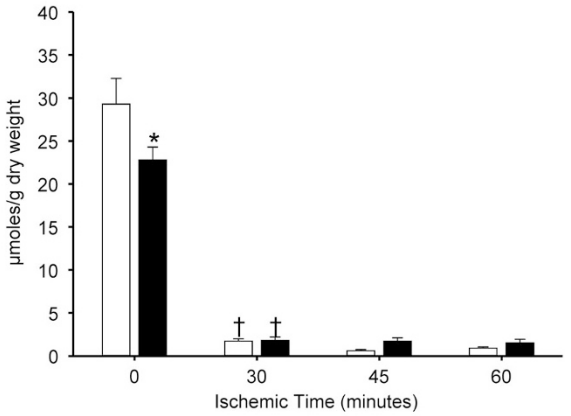

Figure 2. CP levels *in vivo* and at 30, 45, and 60 min of ischemia in newborn male and female piglets. Values expressed as mean ± SEM: *$p < 0.05$ *vs* opposite gender at same time point and †$p < 0.001$ *vs* previous time point within a gender. Males (□); females (■).

however, females exhibited 40% lower ATP content ($p = 0.016$), which was exacerbated after 60 min, where females had 70% lower ATP ($p = 0.009$) compared with males.

Creatine phosphate. *In vivo* CP levels were 22% lower in newborn females compared with males (males: 29.3 ± 3.0; females: 23.0 ± 1.6; $p = 0.043$; Fig. 2). At 30 min of ischemia, CP reserves were completely depleted in both newborn males and females.

Glycogen. Newborn male and female *in vivo* and ischemic myocardial glycogen levels are presented in Figure 3. Females displayed 32% significantly lower *in vivo* myocardial glycogen reserves compared with males (males: 199.8 ± 21.8; females: 133.5 ± 9.7; $p = 0.007$). At 30 min of ischemia, myocardial glycogen reserves declined significantly in both genders ($p < 0.001$); however, with continued ischemia, only the hearts of newborn females showed further significant declines ($p < 0.001$) in glycogen, whereas males reached a plateau after the 30 min mark. By 60 min of ischemia, females broke down a significantly greater amount of myocardial glycogen reserves compared with males (male: 50.4 ± 8.6 *versus* female: 75.4 ± 6.1; $p = 0.028$) and as a result, females

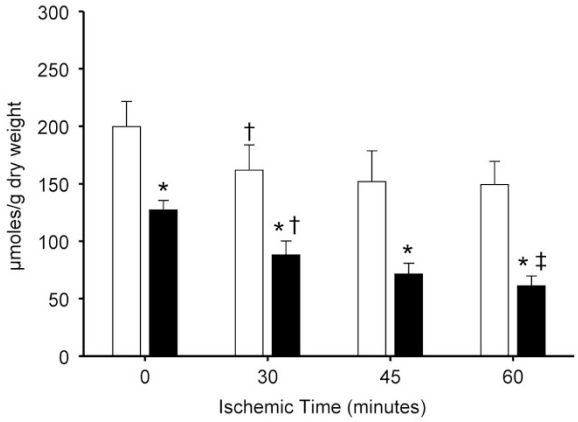

Figure 3. Glycogen levels *in vivo* and at 30, 45, and 60 min of ischemia in newborn male and female piglets. Values expressed as mean ± SEM: $*p < 0.005$ *vs* opposite gender at same time point $\dagger p < 0.001$ *vs* previous time within a gender, and $\ddagger p = 0.001$ *vs* female at 30 min of ischemia. Males (□); females (■).

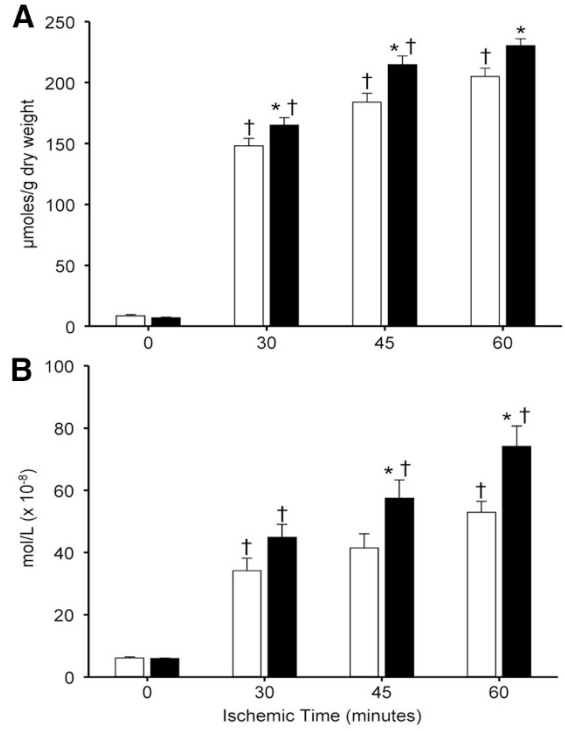

Figure 4. (*A* and *B*) Lactate and H^+ levels *in vivo* and at 15, 30, 45, and 60 min of ischemia in newborn male and female piglets. Values expressed as mean ± SEM: $*p < 0.05$ *vs* opposite gender at same time interval, $\dagger p < 0.03$ *vs* previous time within a gender. Males (□); females (■).

continued to have lower myocardial glycogen throughout the entire ischemic period ($p < 0.003$).

Lactate. *In vivo* myocardial lactate levels were comparable between newborn males and females (males: 8.5 ± 1.2; females: 6.7 ± 0.6; $p = 0.165$; Fig. 4*A*) and were within the normal range for newborn piglets (13). After 30 min of ischemia, despite a significant accumulation ($p < 0.001$) of lactate in both male and female hearts, females accumulated significantly ($p = 0.049$) more lactate compared with males. This difference was exacerbated at 45 min of ischemia ($p < 0.001$) as both sexes continued to significantly ($p < 0.001$) accumulate lactate, after which lactate accumulation in males continued ($p < 0.001$), whereas in females it reached a plateau. Despite this, females still maintained higher lactate levels even at 60 min of ischemia ($p = 0.01$).

Table 2. *Enzyme activity*

Enzyme	Males	Females
CK	99.1 ± 9.7	101.1 ± 6.9
GP	3.3 ± 0.2	3.3 ± 0.1
HK	2.4 ± 0.2	2.2 ± 0.1
PFK	9.1 ± 0.9	9.1 ± 0.7
LDH	48.4 ± 2.5	48.5 ± 3.4

Newborn male and female myocardial enzyme activity (mmoles/g protein/h). Values expressed as mean ± SEM.

LDH, lactate dehydrogenase.

Hydrogen ion. There were no significant differences between newborn male and female *in vivo* H^+ levels (males: 6.1 ± 0.3; females: 5.9 ± 0.2; $p = 0.599$; Fig. 4*B*), and these values were within the normal range for newborn piglets. After 30 min of ischemia, despite a significant increase ($p < 0.001$) in H^+ levels in both males and females, females accumulated 31% greater H^+ compared with males. This sex difference was exacerbated with continued ischemia H^+ ($p < 0.03$) such that after 60 min of ischemia, females demonstrated 45% higher H^+ content ($p = 0.002$) compared with males.

Myocardial enzyme activities. There were no differences in the maximum potential activities of CPK, GP, HK, PFK, and LDH between the newborn male and female left ventricle (Table 2).

DISCUSSION

Previous work demonstrated that in newborns, compared with the right ventricle, the left ventricle had enhanced glycolytic capacity which resulted in greater anaerobic end-product accumulation and lower energy levels during ischemia (25). This study is the first to investigate and characterize sex-specific differences in baseline and ischemic metabolism in this high-risk ventricle of the newborn. The data show newborn females may be at a greater metabolic risk even before a hypoxic or ischemic stress as demonstrated by significantly lower baseline high-energy phosphates (ATP and CP) and significantly lower myocardial glycogen reserves compared with newborn males. Although no sex differences in metabolic enzymatic potential were noted, the newborn female myocardium demonstrated a greater and more rapid accumulation of anaerobic end-products indicating a greater ability to undergo anaerobic glycolysis during ischemia. Despite this, females had lower ischemic ATP levels. Greater and more rapid development of tissue lactate acidosis in the face of lower energy levels during ischemia may place the newborn female LV at greater metabolic risk compared with males, potentially negatively affecting postischemic myocardial functional recovery.

Although no parameter which may reflect ischemic tolerance or postischemic myocardial function was measured in this study, ample evidence from previous metabolic studies have demonstrated the potentially detrimental effects of the

metabolic profiles identified in this study. For example, in the newborn myocardium, lower preischemic high-energy phosphate and glycogen reserves have been associated with lower ATP levels during ischemia, more rapid development of ischemic contracture, and increased postischemic ventricular dysfunction (10,13,26). In children undergoing congenital cardiac repair, slightly lower levels of preischemic ATP (~4 μmol/g dry weight) was also strongly associated with more rapid ATP depletion during ischemia, worse postoperative myocardial function, longer time spent in the intensive care unit (ICU), and longer hospital stays (27). Other investigators have suggested that accumulation of anaerobic end-products during ischemia and not reduced ATP levels is responsible for damage to the myocardium during ischemia and reperfusion (28). The development of lactic acidosis during ischemia has been reported to trigger an ion exchange sequence resulting in Ca^{2+} overload, increased mitochondrial damage and cell death, and myocardial dysfunction after reperfusion (12,29,30). Based on these and other studies, the combination of lower high-energy phosphates, lower anaerobic substrate reserves (glycogen), and greater lactic acidosis during ischemia would place the newborn female myocardium at a metabolic disadvantage, resulting in significantly greater postischemic ventricular dysfunction.

Baseline metabolism. During the perinatal period, LV workloads increase dramatically and result in rapid physiologic hypertrophy of the ventricle (31). In the adult population, both clinical and experimental studies suggest that sex of an individual may influence the response of the myocardium to pressure overload with females developing a greater degree of myocardial hypertrophy in response to similar workloads (32–34). Because increases in myocardial workloads and myocardial hypertrophy are associated with a greater reliance on carbohydrate and glycolytic metabolism (35–37), a greater response of the newborn female myocardium to perinatal changes in systemic workloads may offer a potential explanation for sex-specific differences identified in this study.

Currently, there are no studies in the literature that have reported sex-specific differences in newborn heart metabolism. Several studies in adults, however, suggest that the female myocardium has a greater reliance on glycolytic *versus* oxidative pathways for ATP production (6–8) and that females are more reliant on carbohydrate *versus* fatty acid substrates when compared with males (5). Adult females are also reported to have lower myocardial glycogen (6). Because myocardial glycogen reserves are preferentially oxidized (36,38), and because adult females are less reliant on this pathway, a lower baseline reserve of glycogen is maintained compared with that seen in males. Furthermore, because metabolism of carbohydrates are a less efficient substrate for ATP production compared with fatty acids, the greater glucose reliance of females in part explains lower levels of baseline myocardial high-energy phosphates. If these sex-specific differences in adults are also present in the newborn myocardium, this could offer a potential explanation for lower baseline myocardial glycogen reserves and lower high-energy phosphates (ATP and CP) in newborn females.

Lower baseline energy reserves in the newborn female myocardium may also be influenced by sex-specific differences in cardiac workloads and energy demand. In this study, no sex-specific differences in blood gas, pH, and pressures were noted. Interestingly, newborn female piglets demonstrated significantly higher resting heart rates compared with males. A higher resting heart rate in females has also been reported previously in both the adult and newborn myocardium (39–41). Experimental studies in adults have demonstrated that myocardial energy charge and glycogen reserves decrease in proportion to increasing heart rate (42) and this seems to also be the case in this study, because females demonstrated significantly lower myocardial high-energy phosphate and glycogen reserves.

Response during ischemia. During ischemia, because oxygen availability begins to decline, the myocardium shifts away from oxidative processes and relies on anaerobic glycolysis as the major pathway by which to continue generating ATP. Because there is no blood flow during myocardial ischemia, anaerobic end-products such as lactate and H^+ accumulate and thus serve as an indirect indicator of the degree of anaerobic glycolysis (43). During ischemia, newborn females accumulated a more rapid and greater degree of both lactate and H^+, indicating that they underwent a greater amount of anaerobic glycolysis. In the newborn heart, the more rapid accumulation of anaerobic end-products during ischemia compared with adults has been reported to feedback earlier on glycolytic enzymes such as PFK to slow down or completely inhibit the glycolytic pathway (9,10,44). In this study, cessation of glycolysis occurred earlier in the newborn female myocardium, as demonstrated by lactate accumulation reaching a plateau by 45 min of ischemia, whereas in males, lactate continued to significantly accumulate until 60 min. Greater feedback inhibition of glycolysis in the newborn female myocardium may further reduce the ATP-producing ability and offer a potential explanation for their lower levels of ATP during ischemia. This is confirmed as sex-specific differences in ischemic ATP levels coincided with cessation of glycolysis in the newborn female myocardium.

During ischemia, substrate delivery is impaired and glycogen becomes one of the sole substrates available for metabolism *via* anaerobic glycolysis. Despite having lower levels at baseline, newborn females demonstrated a greater ability to use their glycogen reserves by breaking down over 54% by 60 min of ischemia compared with males who broke down only 25%. These results therefore demonstrate that greater accumulation of lactate in females can also be attributed to sex-specific differences in ability to undergo glycogenolysis during ischemia. It should also be noted however, that both sexes fail to completely use their glycogen reserves by 60 min of ischemia, even in the face of a rapid depletion in myocardial ATP. Incomplete utilization of glycogen reserves during ischemia has been previously reported in the newborn male myocardium (10,11) and is believed to be due to anaerobic end-product feedback inhibition of glycolysis. Interestingly, this study demonstrated that despite greater and more rapid accumulation of anaerobic end-products, the newborn female myocardium had a greater ability to undergo glycogenolysis dur-

ing ischemia suggesting the presence of sex-specific differences in glycolytic enzyme sensitivity to anaerobic end-products in the newborn myocardium. In addition, previous work demonstrated that in newborns, the left ventricle despite having higher glycogen levels than the right ventricle had greater anaerobic end-product accumulation and lower energy levels during ischemia (25). The current work suggests this ischemic profile is exacerbated in females, putting them at even greater risk.

In conclusion, this study is the first to demonstrate that even in the newborn, sex-specific differences exist in myocardial metabolism and response to ischemia. Compared with males, the left ventricle of newborn females demonstrated significantly lower levels of baseline high-energy phosphates and anaerobic substrate reserves (glycogen) which may place them at potential metabolic risk even before oxygen stress. During ischemia, the newborn female myocardium demonstrated a greater ability to use glycogen reserves and accumulated a greater amount of anaerobic end-products, which was associated with earlier inhibition of glycolysis and lower ischemic ATP levels compared males. These results offer one potential explanation for clinical studies that have reported worse outcome of female children during management and after repair of congenital heart disease (1–4). Tailored cardioprotective strategies may therefore be required to help reduce the metabolic risks associated with female sex during clinical management in children.

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