Mechanical and Metabolic Characterization of Ischemic Contracture in the Neonatal Pig Heart

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ABSTRACT

Isolated, paced, isovolumetrically beating piglet hearts ($n = 37$) underwent retrograde aortic perfusion with a crystalloid solution during three periods: 1) baseline (coronary perfusion pressure 60 mm Hg), 2) ischemia (coronary flow 10% of baseline for ≈80 min), and 3) reperfusion (perfusion pressure returned to baseline). In one group of hearts, glycolysis (using ^3H_2O formation from [^3H]glucose) was assessed. During baseline, peak systolic pressure (PSP) was 101.1 ± 5.0 mm Hg, end diastolic pressure (EDP) 4.4 ± 0.5 mm Hg, glycolysis 970.5 ± 65.3 nmol/min/g_{wet}, and myocardial glycogen 234.8 ± 12.0 μmol/g_{dry}. During ischemia, PSP decreased to 23.3 ± 2.7 mm Hg, EDP increased to 12.3 ± 0.7 mm Hg, myocardial glycogen decreased to 181.5 ± 30.3 μmol/g_{dry}, and lactate (≈154 μmol/g_{wet}) and glycerol (≈930 nmol/g_{wet}) were released. Myocardial contracture correlated with a decrease in lactate release. Glycolysis decreased to ≈400 nmol/min/g_{wet} and remained stable, accounting for ≈50% of the lactate produced. During reperfusion, PSP recovered to 79.8 ± 3.5 mm Hg, EDP 6.6 ± 1.7 mm Hg, and glycolysis 1103.9 ± 81 nmol/min/g_{wet}. In a second group of hearts, with similar mechanical responses, glucose oxidation (using $^{14}CO_2$ formation from [^{14}C]glucose) was evaluated. During baseline, glucose oxidation was 165.4 ± 15.9 nmol/min/g_{wet} and correlated closely ($r = 0.957$) with mechanical activity. With ischemia, glucose oxidation decreased to ≈17

nmol/min/g_{wet}, yet accounted for ≈42% of the ATP produced. Upon reperfusion, glucose oxidation returned to baseline values, but now correlated poorly ($r = 0.574$) with mechanical activity. We conclude that for neonatal hearts undergoing severe low-flow ischemia: 1) myocardial contracture is associated with a decline in lactate release, implying impaired lactate production and/or clearance; 2) glycolysis may not fully account for the lactate released, suggesting nonglycolytic sources for energy production; 3) glycerol release is stimulated, indicating hydrolysis of triacylglycerols; 4) glucose oxidation provides an important source of ATP; and 5) glycolysis and glucose oxidation return to baseline values upon reperfusion, despite depressed contractile function, indicating dissociation between mechanical and metabolic recovery. (*Pediatr Res* **38**: 228–236, 1995)

Abbreviations

PSP, peak systolic pressure
EDP, end diastolic pressure
(+)dP/dt$_{max}$, maximum rate of rise of pressure with respect to time
Pao$_2$, partial pressure oxygen (arterial)
Pvo$_2$, partial pressure oxygen (venous)
MVo$_2$, myocardial oxygen consumption

Myocardial contracture represents a state of decreased diastolic compliance of the ventricle secondary to a depletion of high energy stores in the region of the contractile apparatus (1). With ischemia, contracture occurs as a consequence of myocardial underperfusion, which results in inadequate substrate delivery and impaired ATP production. Insufficient ATP leads to a progressive increase in the number of myocin-actin complexes that are unable to dissociate and to derangements in resting cytosolic calcium levels (2).

Previous investigations of myocardial contracture in neonates have focused on immature hearts in an arrested state with zero coronary flow (3, 4). Little is known about myocardial contracture in the setting of low-flow ischemia, where hearts are beating, and there is washout of metabolites. Additionally, the modalities of energy production and utilization associated with contracture are poorly understood, yet these processes ultimately determine myocardial viability. An understanding of these processes in neonatal hearts has significant clinical ramifications, because contracture is thought to represent a precursor to an irreversible myocardial injury (5).

The objectives of the present investigation were to study myocardial contracture in isolated, isovolumetrically beating, neonatal pig hearts subjected to normothermic, low-flow isch-

emia. Mechanical and metabolic responses were evaluated in hearts perfused with a solution that favored glucose utilization, because glucose has been recognized as an important substrate for the prevention of ischemic contracture in mature hearts (6) and has been used clinically as a resuscitative agent to promote recovery of myocardial contractile function after ischemic injury (7). We speculated that contracture might be associated with an alteration in substrate metabolism, through lactate release, because lactate is an end product of anaerobiasis. Functional recovery after ischemic contracture was also investigated in reperfused hearts.

METHODS

Preparation of perfusate. The perfusate (37°C) consisted of insulin (100 μU/mL); the following salts in mM: NaCl, 118; KCl, 4.7; MgSO$_4$, 2.4; KH$_2$PO$_4$, 1.2; NaHCO$_3$, 25; CaCl$_2$, 2.4; NaEDTA, 0.1 mM; glucose 5.5 mM; and 2% BSA (Fraction V, RIA grade, >98% purity, U.S. Biochemical Corp., Cleveland, OH). Supplementary fatty acids were not included in the perfusate. The pH of the solution was adjusted to 7.4–7.45 by titration with 1 N NaOH.

Excision of hearts. Pigs, less than 3 d of age, were used for these studies, because they compare favorably in size with those of humans of similar maturity. Piglets were anesthetized with sodium pentobarbital (25 mg/kg i.p.), anticoagulated with sodium heparin (1000 U/kg i.v.), and mechanically ventilated via a tracheostomy. A midline sternotomy was then performed to expose the heart and great vessels.

A catheter was advanced into the aortic root via the right carotid artery, and the remaining aortic arch vessels were ligated. After ligation of the cavae, incisions were made in the main pulmonary artery and pulmonary veins to decompress the heart. The transverse aortic arch was then cross-clamped. Topical cooling of the heart with ice-cold saline was begun, while simultaneously 4°C cardioplegic solution ($\approx$30 mL/kg body weight) was administered over $\approx$2 min, via the aortic root catheter. Cardioplegic solution was prepared from a stock solution of 5% dextrose and 0.45% NaCl, to which KCl and NaHCO$_3$ were added to yield final concentrations of 16 mEq/L each (8). The pH of the cardioplegic solutions at 4°C was $\approx$7.8. After cardioplegia, the hearts were excised and immediately placed in ice-cold Krebs-Henseleit solution.

Perfusion of isolated hearts. The aorta was cannulated, and each heart underwent retrograde aortic perfusion using the modified Langendorff system described previously (9–11). The time that elapsed between excision of hearts and initiation of retrograde aortic perfusion was less than 5 min. The pulmonary artery was cannulated, and the pulmonary veins were ligated. The coronary perfusion pressure was continuously monitored with a pressure transducer attached to a side port just above the heart. In this preparation, coronary venous return eggressed from the pulmonary artery cannula. Hearts were paced at 180 bpm via the right ventricle, so that ventricular contractions would be maintained at a constant rate when studying low-flow ischemia, which could have affected atrioventricular conduction. The arterial perfusate was preoxygenated with a pediatric oxygenator (Bently), using a mixture of

95% O$_2$-5% CO$_2$, and then passed through the Langendorff column. This procedure yielded a final arterial PO$_2$ of $\approx$73 kPa ($\approx$550 mm Hg). A "nonrecirculating system" was used to ensure delivery of steady state concentrations of substrates to the heart, and to avoid effects from unrecognized metabolites.

Coronary flow and mechanical function. Coronary flow was measured by timed collections of the pulmonary artery (coronary venous) effluent. Left ventricular isovolumic PSP, the maximum rate of rise of systolic pressure with respect to time (+dP/dt$_{max}$) and EDP were recorded continuously via a fluid-filled balloon catheter in the left ventricular chamber. The EDP was adjusted by changing the volume of fluid in the balloon. Any fluid within the ventricular chamber, *e.g.* from the Thebesian circulation, was vented through an apical stab wound. This maneuver reduces the possibility that a fluid collection around the balloon could alter EDP (9–11).

Metabolic function. Lactate release was studied as a means of characterizing oxygen insufficiency. Lactate concentrations were measured on arterial and venous perfusate samples (12). Rates of lactate release (μmol/min/g$_{wet}$) were calculated from: the arteriovenous lactate concentration difference and coronary flow. Because the arterial perfusate contained no lactate, any lactate detected in the coronary venous effluent was lactate that was released by the heart.

Glycolysis from exogenously supplied glucose was estimated by measuring tritiated water (^{3}H$_2$O) production (released at the phosphoglucoseisomerase step) from D-2[^{3}H(N)]-glucose (100 μCi/L), according to the techniques originally described by Neely *et al.* (13), and Rovetto *et al.* (14). The ^{3}H$_2$O was separated from [^{3}H]glucose using columns containing Dowex 1X4 anion exchange resin (200–400 mesh) suspended in 0.2 M potassium tetraborate. A 0.5-mL volume of perfusate was added to the column and eluted into scintillation vials with 1.5 mL of deionized H$_2$O. The column was then rinsed with an additional 2.0 mL of H$_2$O, which was collected in a separate scintillation vial. After addition of Eco-Lite scintillant (ICN Biochemicals, Costa Mesa, CA), the vials were counted in a scintillation counter, with the window set at 0–300 nm. The counts (minus background) from both vials were added to give the total counts for the sample. Rates of glycolysis (nmol/min/g$_{wet}$) were calculated from the measured specific activity of the [^{3}H]glucose in the perfusate; the arteriovenous ^{3}H$_2$O production difference and the coronary flow.

Myocardial glycogen content was measured in hearts that were freeze-clamped using aluminum blocks (12 $\times$ 8.5 $\times$ 2.5 cm) precooled with liquid nitrogen. Glycogen was extracted by homogenizing ventricular tissue with 1 N perchloric acid, followed by neutralization with 5 N KOH. The extracts were then incubated with amyloglucosidase, and the liberated glucose was measured fluorometrically (15).

Oxidation of exogenously supplied glucose was estimated by measuring ^{14}CO$_2$ production (liberated at pyruvate dehydrogenase and the Krebs cycle) from [U-^{14}C]glucose (100 μCi/L), using the method we have described previously (9). A 3-mL sample of perfusate was obtained anaerobically, and injected into a stoppered Erlenmeyer flask containing 1 N HCl (to release CO$_2$) and a center well with Hyamine hydroxide (to collect CO$_2$). After 12 h of incubation (45°C), the wells were

placed in Ready Organic scintillant, and the $^{14}CO_2$ determined by counting with the window set at 397–655 nm. Rates of glucose oxidation ($nmol/min/g_{wet}$) were calculated from: the measured specific activity of [^{14}C]glucose in the perfusate; the arteriovenous $^{14}CO_2$ production difference and the coronary flow. The contribution of glucose oxidation to oxygen consumption was calculated by assuming 6 mol of oxygen/mol glucose.

Myocardial oxygen consumption (MVo_2, $\mu mol\ O_2/min/g_{wet}$) was calculated as ($Pao_2 - Pvo_2$) × C × coronary flow, where Pao_2 and Pvo_2 refer to the arterial and venous partial pressures of oxygen (kPa), respectively, and where C = (0.23 $\mu L\ O_2/mL/kP_a$)/(22.4 $\mu L\ O_2/\mu mol$). Samples for Po_2 were obtained simultaneously from the aortic perfusion line and pulmonary artery cannula. Oxygen partial pressures were measured directly with a blood gas analyzer (Corning, Medfield, MA).

Average rates of ATP production from glycolysis were calculated by assuming 2 mol ATP/mol exogenous glucose. The ATP production from subsequent oxidative phosphorylation of glucose was calculated by assuming 34 mol ATP/mol glucose.

Glycerol release was used to assess lipolysis and to evaluate the clearance by the heart of another metabolite besides lactate. Inasmuch as supplementary fatty acids were not included in the perfusate, endogenous myocardial triglycerides became the primary source of lipids available for metabolism. Triglycerides can be hydrolized to glycerol and FFA, and heart tissue is deficient in glycerol kinase (16). Thus, glycerol release can be used as an indirect marker for hydrolysis of triglycerides. Glycerol concentrations were measured on arterial and venous perfusate samples, using a fluorometric assay (17). Rates of glycerol release ($nmol/min/g_{wet}$) were determined from the arteriovenous glycerol concentration difference and coronary flow.

EXPERIMENTAL DESIGN

Normal oxygen delivery (control group). These experiments were performed to demonstrate that these hearts (6–12 g) exhibited prolonged stability under conditions of normal (*i.e.* adequate) oxygen delivery. Hearts from 11 pigs were excised as previously described. After initiation of retrograde aortic perfusion, the hearts were allowed to stabilize for ≈20 min with a coronary perfusion pressure of 60 mm Hg, which is the *in vivo* aortic diastolic pressure measured in piglets (18). The left ventricular EDP was set at ≈5 mm Hg, a normal EDP for a neonate. Typically, the volume of fluid in the balloon, or preload, was ≈0.07 mL/g_{wet}. Each heart underwent control perfusion for an additional 100 min, which was the average time for the low-flow ischemia protocol. Left ventricular PSP, +dP/dt$_{max}$ and EDP, coronary flow, lactate release, and MVo_2 were measured at 10-min intervals. Seven of the 11 hearts were freeze-clamped at the end of this perfusion period for the measurement of myocardial glycogen contents.

Four additional hearts underwent control perfusion for only 20 min after the initial stabilization period. This perfusion period corresponded to the time of perfusion before inducing

ischemia, in the low-flow ischemia protocol. These hearts were freeze-clamped at the end of this period for the measurement of baseline (preischemic) myocardial glycogen contents.

Low-flow ischemia (group I). These experiments were performed to determine mechanical responses, measure lactate release, estimate glycolysis from exogenously supplied glucose, and assess myocardial glycogen contents. Hearts from six pigs were excised as described above. After initiation of retrograde aortic perfusion, the coronary perfusion pressure was fixed at 60 mm Hg, and the left ventricular EDP at ≈5 mm Hg. Because the volume of fluid in the balloon was held fixed, subsequent changes in EDP reflected alterations in left ventricular diastolic compliance. Hearts were then allowed to stabilize for ≈20 min, during which time [^3H]glucose was added to the perfusate. During this period of time, ^3H_2O production reached a steady state, indicating that the isotopically labeled glucose had equilibrated with the endogenous myocardial glucose pool.

After stabilization, each heart underwent three consecutive perfusion periods: 1) baseline, 2) normothermic (37°C) low-flow ischemia, and 3) reperfusion. During baseline, which lasted 20 min, hearts were perfused at a constant coronary perfusion pressure of 60 mm Hg, and with an EDP of ≈5 mm Hg. During low-flow ischemia, the coronary flow was abruptly reduced to 10% of the baseline value, using the constant flow system described previously (11). A fixed coronary flow was used to produce low-flow ischemia, so that global oxygen delivery would remain constant even as myocardial contracture developed. The onset of contracture was defined by a progressive rise in left ventricular EDP. Ischemia was continued until the EDP increased ≈2-fold above the baseline value, to ensure that contracture was sustained.

Hearts were reperfused by abruptly restoring coronary perfusion pressure to the baseline value of 60 mm Hg and allowing coronary flow to run freely. Because perfusion pressure was again held constant, coronary flow and thus clearance of metabolites was determined by autoregulation. Reperfusion was continued until the PSP and +dP/dt$_{max}$ stabilized (≈20 min). Reperfusion was included in this protocol primarily to demonstrate that these piglet hearts recovered function after the severe ischemic challenge.

The following measurements were taken at 5-min intervals: left ventricular PSP, +dP/dt$_{max}$ and EDP, coronary flow, lactate and glycerol release, and ^3H_2O production from exogenously supplied [^3H]glucose. The bolus of lactate released upon reperfusion was determined by collecting four timed samples of coronary venous effluent during the first ≈90 s of reperfusion and analyzing these samples for lactate content. The bolus of glycerol release was determined in a similar fashion. Six additional hearts were subjected to the same low-flow ischemia protocol but freeze-clamped at the conclusion of ischemia, to measure glycogen contents.

Low-flow ischemia (group II). These experiments were conducted to assess aerobic metabolism by measuring oxidation of exogenously supplied glucose and MVo_2. Correlations were sought between glucose oxidation and mechanical activity and MVo_2. For these studies, six hearts were subjected to the low-flow ischemia protocol. However, during this stabilization period, [^{14}C]glucose (instead of [^3H]glucose) was added

to the perfusate. Here again, we found that ≈20 min of heart perfusion was sufficient for the exogenously labeled glucose to equilibrate with the endogenous myocardial glucose pool, as reflected by a steady state of $^{14}CO_2$ production. To compare pre- and postischemic metabolic responses, rates of glucose oxidation and MVo_2 were normalized to mechanical activity by dividing these metabolic rates by [(PSP-EDP) × HR].

Low-flow ischemia (group III). Glycerol release was studied in the group I hearts and in four additional hearts that were subjected to the low-flow ischemia protocol.

Data and statistical analysis. Data values are expressed as means ± SEM. Data obtained during ischemia and reperfusion were analyzed and compared with baseline, using a one-way analysis of variance randomized block design for repeated measures, followed by Dunnett's and/or Newman-Keuls comparison procedure. Differences were considered significant with values of $p < 0.05$.

RESULTS

Normal Oxygen Delivery (Control Group)

The parameters measured at 20 and 100 min of perfusion for the control group are shown in Table 1. The myocardial glycogen contents with control perfusion are similar to those reported by Werner *et al.* (18) for neonatal pig hearts undergoing Langendorff perfusion with a crystalloid solution containing glucose as the sole substrate. These results demonstrate that hearts excised and perfused, as described above, exhibited mechanical and metabolic stability under conditions of normal oxygen availability.

Low-Flow Ischemia (Group I)

Coronary flow and mechanical function. Figure 1 shows the coronary flow and EDP for the three perfusion periods. During baseline, coronary flow averaged 8.7 ± 0.7 mL/min/
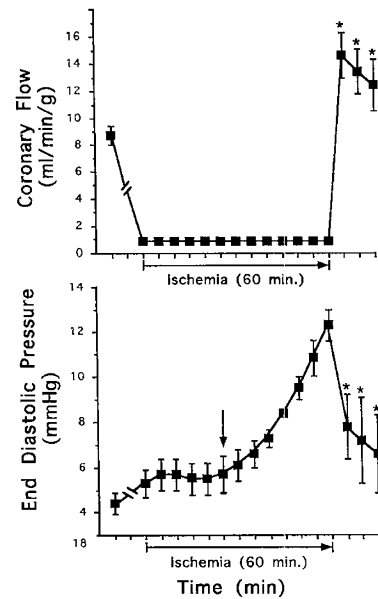

Figure 1. Values of coronary flow, in mL/min/g_{wet} (top graph), and left ventricular end diastolic pressure, in mm Hg (bottom graph), during the three perfusion periods, for the six hearts in group I. The first point represents the average value of the measured parameter during the baseline period. The next 13 points, designated by the *horizonal arrow* (ischemia), represent the values, at 5-min intervals, during the last 60 min of low-flow ischemia. The last three points represent the values during 15 min of reperfusion. Values are expressed as means ± SEM. In the bottom graph, the *vertical arrow* designates the onset of myocardial contracture, as assessed by the rise in end diastolic pressure (see text). Myocardial contracture occurred after 41.7 ± 4.4 min of low-flow ischemia. *Asterisks* indicate the reperfusion values that are statistically different from baseline ($p < 0.05$).

g_{wet}. During ischemia, coronary flow was held constant at 0.87 ± 0.06 mL/min/g_{wet}. Upon reperfusion, coronary flow increased ≈70% above baseline and then decreased toward baseline. During baseline, EDP averaged 4.4 ± 0.5 mm Hg. The volume of fluid in the balloon was 0.069 ± 0.002 mL/g_{wet}. With the induction of low-flow ischemia, the EDP decreased slightly below baseline, reflecting the effect of the reduced coronary flow on left ventricular diastolic compliance (19) and then increased, but remained stable. After a period of stability, the EDP for each heart exhibited a progressive rise, indicating the onset of contracture. The time at which this rise occurred, defined the time for the onset of contracure for each heart. At the conclusion of ischemia, the EDP was 12.3 ± 0.7 mm Hg. The duration of ischemia for the onset of contracture was 41.7 ± 4.4 min, and the total period of ischemia was 78.2 ± 4.6 min. During reperfusion, the EDP decreased toward baseline. Unless stated otherwise, the data have been combined so that only values of parameters corresponding to the last 60 min of low-flow ischemia for each heart are shown.

Figure 2 shows the PSP and +dP/dt_{max}. During baseline, PSP averaged 101.1 ± 5.0 mm Hg. With the induction of low-flow ischemia, PSP abruptly decreased to ≈20 mm Hg, and remained stable. Toward the end of ischemia, PSP tended to increase slightly, reflecting the rise in EDP. With reperfusion, PSP recovered to ≈80% of baseline. During baseline, +dP/dt_{max} averaged 1103.0 ± 80.6 mm Hg/s. The pattern
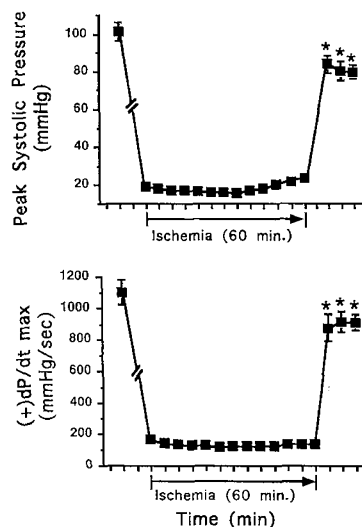
Table 1. *Parameters measured in 11 hearts at 20 and 100 min of control perfusion*

Time (min)	20	100
PSP (mm Hg)	111.7 ± 4.5	106.1 ± 3.7
EDP (mm Hg)	4.2 ± 0.4	5.1 ± 0.7
(+)dP/dt_{max} (mm Hg/s)	1234.0 ± 52.3	1164.0 ± 42.2
Coronary flow (mL/min/g_{wet})*	7.9 ± 0.4	8.3 ± 0.6
Lactate release (μmol/min/g_{wet})	0.62 ± 0.07	0.64 ± 0.09
MVo_2 (μmol/min/g_{wet})	3.22 ± 0.15	3.06 ± 0.17
Glycogen (μmol glucose equivalent/g_{dry})†	234.8 ± 12.0‡	224.6 ± 23.3¶

Values are expressed as means ± SEM.

* Coronary flow, MVo_2, and lactate are expressed per gram wet (g_{wet}) heart weight. Since hearts were freeze-clamped, total wet heart weights were estimated using the regression formula

Heart weight (g) = [0.0065 × body weight (g)] − 0.298.

† Glycogen = myocardial glycogen content.
‡ Measured in four additional hearts freeze clamped after 20 min of perfusion.
¶ Measured in 7 of the 11 hearts freeze clamped after 100 min of perfusion.

Figure 2. Values of left ventricular peak systolic pressure, in mm Hg (top graph), and $(+)dP/dt_{max}$, in mm Hg/s (bottom graph), during the three perfusion periods, for the six hearts in group I. The points shown represent the same time periods as in Figure 1. Values are expressed as means $\pm$ SEM. *Asterisks* indicate the reperfusion values that are statistically different from baseline ($p < 0.05$).

observed for $+dP/dt_{max}$ was similar to that of PSP, except that $+dP/dt_{max}$ did not rise at the end of ischemia.

Lactate release. Figure 3 (top graph) shows rates of lactate release. During baseline, lactate release averaged 0.71 ± 0.11 $\mu mol/min/g_{wet}$. With the induction of low-flow ischemia, lactate release increased initially to 2.42 ± 0.09 and then stabilized at ≈ 2.3 $\mu mol/min/g_{wet}$. After a period of stability, lactate release for each heart began to decline. At the conclusion of ischemia, lactate release was 1.37 ± 0.11 $\mu mol/min/g_{wet}$. Upon reperfusion, a large bolus of lactate was detected, which represented lactate formed during the previous period of ischemia. Lactate release then returned to baseline within 5 min of reperfusion. The total lactate produced as a consequence of ischemia (calculated by integrating the area under the lactate
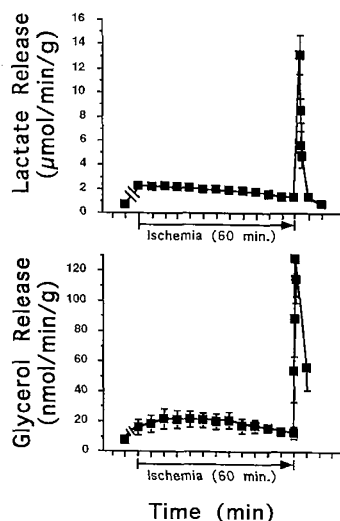

Figure 3. Rates of lactate release, in $\mu mol/min/g_{wet}$ (top graph), for the six hearts in group I. Rates of glycerol release, in $nmol/min/g_{wet}$ (bottom graph), for the four hearts in group III. Values are expressed as means $\pm$ SEM.

release curve for ischemia and the bolus associated with reperfusion) was 154.2 ± 4.6 $\mu mol/g_{wet}$.

Correlation Between EDP and Lactate Release. Figure 4 shows simultaneous values of the left ventricular EDP and lactate release during the last 60 min of ischemia. The rise in EDP, indicating the onset of contracture (*vertical arrow*) and the decline in lactate release, began at approximately the same time. Thus, contracture in the neonatal pig heart was associated with a decline in lactate release.

Glycolysis. Figure 5 (top graph) shows rates of glycolysis from exogenously supplied glucose. During baseline, glycolysis averaged 970.5 ± 65.3 $nmol/min/g_{wet}$. With the induction of low-flow ischemia, glycolysis abruptly decreased to ≈ 400 $nmol/min/g_{wet}$ and remained stable. Glycolysis in our piglet hearts returned to baseline values within 5 min of reperfusion. We observed no significant decline in glycolysis in any heart during the entire period of ischemia, which ranged from 68–98 min, despite the development of contracture.

In the six hearts freeze-clamped at the conclusion of ischemia, the myocardial glycogen content averaged 181.5 ± 30.3 μmol of glucose equivalent/g_{dry}. This value was not statistically different from the baseline (or preischemic) myocardial glycogen content (234.8 ± 12.0 μmol of glucose equivalent/g_{dry}). The variability in the glycogen contents at the conclusion of ischemia precluded an accurate assessment of glycogenolysis. Nevertheless, no net glycogen turnover was detected as a consequence of ischemia.

Low-Flow Ischemia (Group II)

Mechanical function. These hearts exhibited almost identical levels of mechanical performance, times for the onset of contracture (42.6 ± 3.3 min), and total period of ischemia (76.3 ± 7.5 min) to the hearts in group I.

Glucose oxidation. Figure 5 (bottom graph) shows rates of glucose oxidation from exogenously supplied glucose. During baseline, glucose oxidation averaged 165.4 ± 15.9 $nmol/min/g_{wet}$, which accounted for $\approx 48\%$ of the oxygen consumed and correlated closely with both mechanical activity ($r = 0.957$), and MVo_2 ($r = 0.991$). With the induction of low-flow ischemia, glucose oxidation abruptly decreased to ≈ 17 $nmol/min/g_{wet}$, reflecting the corresponding decrease in developed pressure. Glucose oxidation initially remained stable and then

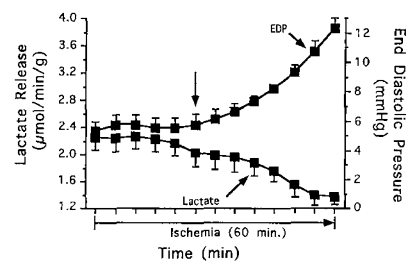**Figure 4.** Simultaneous values of lactate release (bottom curve) and left ventricular end diastolic pressure (top curve), at 5-min intervals, during the last 60 min of ischemia. The scale for lactate release is on the left-sided ordinate, and the scale for end diastolic pressure is on the right. The *vertical arrow* designates the onset of myocardial contracture. Values are expressed as means $\pm$ SEM.

began to decline, at approximately the time at which contracture developed ($p < 0.01$). Glucose oxidation accounted for ≈39% of the oxygen consumed early in ischemia, but declined to ≈28% by the end of ischemia. During reperfusion, glucose oxidation returned to baseline values within 10 min; however, glucose oxidation now correlated poorly with both mechanical activity ($r = 0.574$), and MVo_2 ($r = 0.327$).

$MVo2$, glucose oxidation and glycolysis normalized to mechanical activity. Myocardial oxygen consumption and glucose oxidation normalized to mechanical activity are shown in Figure 6, A and B, for the three perfusion periods. For comparison, the glycolytic rates for the group I hearts were also indexed to mechanical activity (Fig. 6C). A greater amount of oxygen was consumed per mm Hg of pressure development during ischemia than during baseline ($p < 0.05$). Furthermore, for all the hearts studied, the instantaneous value of MVo_2 indexed to mechanical activity increased ≈40% from the beginning of ischemia to contracture. Myocardial oxygen consumption normalized to mechanical activity also remained elevated during reperfusion (although not statistically different from baseline). Glycolysis relative to mechanical activity markedly increased during ischemia compared with baseline ($p < 0.05$), whereas glucose oxidation became enhanced upon reperfusion ($p < 0.05$).

ATP Production Associated with Glucose Utilization

Average rates of ATP production associated with glycolysis (group I) and glucose oxidation (group II) were calculated. These results are presented in Table 2. During baseline, glycolysis accounted for 26% and glucose oxidation 74% of total calculated ATP from exogenous glucose utilization. In contrast, during ischemia, these percentages changed to 58 and 42%, respectively. During reperfusion, the percentages returned to baseline values. Our baseline percentages are somewhat different from those (14 and 86%, respectively) calculated
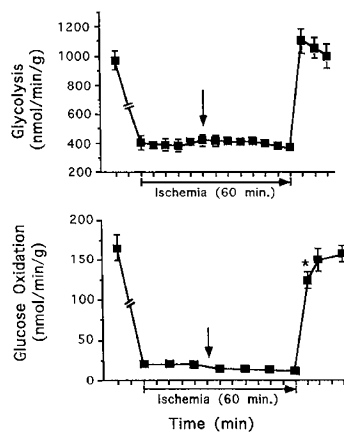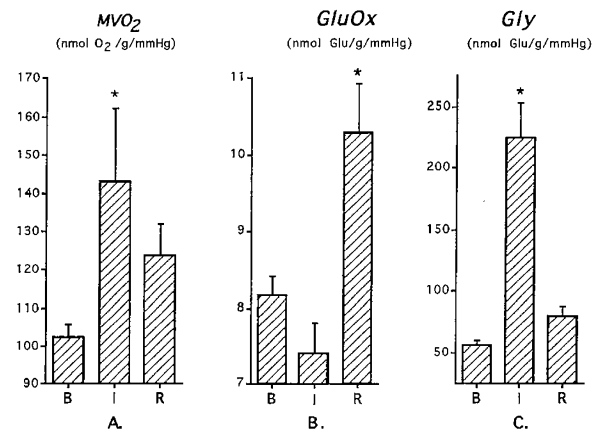

Figure 6. Metabolic rates to mechanical activity, *i.e.*

$$\frac{\text{Metabolic rate (nmol/min/g}_{\text{wet}}) \times 10^3}{(\text{PSP} - \text{EDP}) \times \text{HR (mm Hg/min)}}$$

A, Myocardial oxygen consumption to mechanical activity (MVo_2); *B*, glucose oxidation to mechanical activity ($GluOx$); and *C*, glycolysis to mechanical activity (Gly), are shown for the three perfusion periods (B = baseline, I = ischemia, and R = reperfusion). *Asterisks* indicate that, during ischemia, MVo_2 and Gly are enhanced relative to baseline ($p < 0.05$); whereas, during reperfusion, GluOx becomes enhanced ($p < 0.05$). The remaining metabolic rates are not statistically different from baseline.

from the results of Neely *et al.* (20) for isolated rat hearts perfused with a buffer solution, reflecting the higher oxidation rates in mature hearts.

Low-Flow Ischemia (Group III)

Figure 3 (bottom graph) shows rates of glycerol release for the four additional hearts subjected to the low-flow ischemia protocol. During baseline, glycerol release averaged 7.8 ± 2.3 nmol/min/g$_{\text{wet}}$. Within 5 min of ischemia, glycerol release was stimulated, and it stabilized at ≈20 nmol/min/g $_{\text{wet}}$. With the onset of contracture, glycerol release began to decline, as was observed for lactate release. At the conclusion of ischemia, glycerol release was 12.7 ± 4.2 nmol/min/g$_{\text{wet}}$, a comparable decline as with lactate (Fig. 3, top graph) Upon reperfusion, a bolus of glycerol was detected, which likely represented glycerol formed during the previous period of ischemia. The hearts in group I exhibited a similar pattern of glycerol release.

DISCUSSION

In these studies, we investigated mechanical and metabolic responses in neonatal pig hearts subjected to low-flow ischemia. Hearts were perfused with a crystalloid solution so that severe oxygen insufficiency would be created during myocardial underperfusion, and thus contracture would be a feature of these hearts. These hearts exhibited stability with this preparation under conditions of normal oxygen availability. When hearts were subjected to reduced oxygen delivery (10% of baseline), however, PSP and $+dP/dt_{\text{max}}$ decreased abruptly, but then remained stable. Oxygen extraction increased from a mean value of 49% during baseline to 81% with ischemia, and lactate release increased ≈3–4 fold. After ≈40 min of myo-

Figure 5. Rates of glycolysis, in nmol/min/g$_{\text{wet}}$ (top graph), during the three perfusion periods, for the six hearts in group I. Rates of glucose oxidation, in nmol/min/g$_{\text{wet}}$ (bottom graph), during the three perfusion periods, for the six hearts in group II. *Vertical arrow* designates the onset of myocardial contracture for each group of hearts. Values are expressed as means ± SEM. *Asterisks* indicate the reperfusion values that are statistically different from baseline ($p < 0.05$).

Table 2. *Calculated average rates of ATP production from exogenously supplied glucose*

Mode of ATP production	ATP production (nmol/min/g_{wet})			Percent of total calculated ATP		
	Baseline	Ischemia	Reperfusion	Baseline	Ischemia	Reperfusion
Glycolysis	1941	800	2095	26	58	29
Glucose oxidation	5624	568	5243	74	42	71
Total ATP	7565	1368	7338	100	100	100

These rates were calculated assuming: 2 mol of ATP/mol of exogenous glucose (glycolysis) and 34 mol of ATP/mol of exogenous glucose (glucose oxidation).

cardial underperfusion, hearts developed contracture, as evidenced by a progressive rise in left ventricular EDP.

The mechanisms responsible for reduced contractility and the development of contracture with ischemia are incompletely understood. Reduced oxygen delivery can impair ATP formation, resulting in net ATP hydrolysis. This process leads to depletion of ATP and accumulation of inorganic phosphate (and H^+), which are known to depress myofibril function (21). Obviously, these mechanisms are interrelated, because pH can alter ATP hydrolysis, and, conversely, ATP is required for pH regulation. Furthermore, recent work by Tani and Neely (22) in mature rat hearts suggests that Na^+-H^+ and Na^+-Ca^{2+} exchange may be involved in ischemic myocardial dysfunction. It has been proposed that intracellular H^+ exchanges with Na^+ during ischemia. The corresponding increase in intracellular Na^+ activates the Na^+-Ca^{2+} pump, resulting in excessive Ca^{2+} uptake and altered diastolic chamber compliance. The importance of such ion fluxes in the neonatal heart, however, remains to be elucidated.

Although the neonatal hearts used in this study developed contracture during ischemia, PSP and $+dP/dt_{max}$ returned to ≈80% of baseline values upon reperfusion. These results are consistent with our previous findings (23) and the recent reports by Matherne *et al.* (24) and Downing and Chen (25) that immature hearts demonstrate tolerance to low-flow ischemia and exhibit good recovery of contractile function upon reperfusion. Functional recovery after an ischemic challenge is believed to reflect an enhanced capacity to maintain ATP stores and H^+ balance during the previous ischemic episode. Recent work by Matherne *et al.* (24), using ^{31}P-NMR spectroscopy, has shown that neonatal rabbit hearts are more resistant to global ischemia than are their adult counterparts and that intracellular ATP and pH are maintained more effectively in the immature myocardium.

In contrast, other investigators have suggested that the neonatal heart may be particularly susceptible to ischemic injury (4, 26, 27). It has been postulated that the enhanced capacity of the immature heart for anaerobic glycolysis leads to rapid accumulation of lactate (and H^+), which accelerates myocardial injury during oxygen insufficiency (4). However, in these studies, neonatal hearts were subjected to zero coronary flow. In contrast, our model allows for continuous perfusion of the myocardium. A minimal level of coronary flow may be required to provide washout of metabolites and thus prevent a critical buildup of lactate.

An important finding in the present study was that the increase in ventricular diastolic stiffness (contracture) during ischemia in these neonatal hearts occurred in conjunction with a decline in lactate release. This decline in lactate release may

reflect a decrease in lactate production. Previous investigations, for example, have demonstrated that glycolytic flux, and thus lactate production, may become inhibited during ischemia by an accumulation of metabolites and H^+ (14, 28). Recently, Kingsley *et al.* (29), using ^{31}P-NMR spectroscopy to measure intracellular pH, demonstrated that contracture in mature rat hearts correlated with a plateau in intracellular pH. They suggested that contracture, at least in the arrested heart, may be associated with a cessation of anaerobic glycolysis. With low-flow ischemia, however, there can be washout of H^+; thus, a decline or cessation in H^+ formation would be expected to be associated with a rise, rather than a plateau, in intracellular pH. Because, in our hearts, the rate of glycolysis from exogenously supplied glucose was stable during ischemia, a decrease in lactate and/or H^+ production with contracture may be related to: 1) a decrease in glycogen utilization and 2) nonglycolytic pathways for lactate production. In the first case, we found no net turnover of glycogen, based on changes in myocardial glycogen contents. In the second case, however, nonglycolytic pathways have been identified for anaerobic energy production, and are discussed below (30).

The decline in lactate release during contracture may also be related to impaired lactate clearance. Stein *et al.* (31) have shown that intramyocardial diastolic pressures exceed intracavity diastolic pressures in the left ventricle of the normal dog heart. It is probable that intramyocardial diastolic pressures exceeded intraventricular balloon diastolic pressures in these piglet hearts. Moreover, contracture and edema may have augmented intramyocardial diastolic pressures during the low-flow state. Because coronary extravascular compression is directly related to intramyocardial pressure, we speculate that lactate clearance may have been further impaired by the onset of contracture. The decline in both glycerol and lactate release during contracture is consistent with the notion of impaired clearance. Inasmuch as coronary flow was held constant, however, a compression of the coronary vasculature would be reflected in a redistribution of flow, probably from endocardium toward epicardium.

In the present study, glycolysis was investigated under baseline conditions and during the ischemic challenge. Our baseline rates of glycolysis are similar to those reported by Werner *et al.* (18, 32) for neonatal pig hearts, when wet to dry heart weight ratios are taken into account. With the induction of low-flow ischemia, glycolysis decreased ≈50–60% from baseline, which is comparable to the reduction in glycolysis observed by Neely *et al.* (20) for isolated mature rat hearts subjected to severe ischemia. Moreover, the onset of myocardial contracture in our piglet hearts was associated with stable rates of glycolysis. Thus, contracture was not caused by a

decline in exogenous glucose utilization *per se*. However, ATP stores in the region of the contractile apparatus probably became depleted by $\approx$40 min of ischemia, and it is conceivable that contracture may have occurred as a consequence of an insufficient rate of glycolysis. Recently, Owen *et al.* (6) and Eberlie *et al.* (33) have shown that a minimal rate of ATP production from glycolysis ($\approx$2.0 μmol ATP/min/g_{wet}) was required to prevent ischemic contracture in mature hearts. In our experiments, we estimate that the rate of ATP production from glycolysis during ischemia was $\approx$0.8 μmol ATP/min/g_{wet}. Contracture in these neonatal hearts, therefore, may have been related to an inadequate rate of ongoing, glycolytic ATP production.

The present study also suggests that net lactate production ($\approx$154 μmol/g_{wet}) may not fully be accounted for by glycolysis from exogenously supplied glucose. The net lactate ascribed to glucose was $\approx$75 μmol/g_{wet}, assuming 2 mol of lactate per mol of glucose. Thus, only $\approx$50% of the measured lactate during ischemia was accounted for by glucose utilization. One possibility for this apparent discrepancy may lie in an underestimate of the radioisotopically determined rate of glycolysis during ischemia. For example, unlabeled glucose from glycogenolysis could dilute the pool of labeled glucose being taken up from the perfusate, resulting in a lower measured rate of glycolysis. However, in our experiments, hearts were equilibrated with [^{3}H]glucose and insulin for $\approx$50 min before inducing ischemia. Thus, a sufficient time was allowed for incorporation of labeled glucose into glycogen and for the perfusate glucose radioactivity to equilibrate with that of the endogenous glucose pool (34, 35). This issue, however, deserves additional investigation.

Recent studies by Wittnich *et al.* (3) on globally ischemic neonatal pig hearts also suggest that net lactate production may not be accounted for by glycogenolysis. In these studies, piglet hearts were subjected to no-flow arrest, until the development of contracture, by immersing hearts in a glucose-free buffer solution. Based on their measured changes in myocardial glycogen ($\approx$6 μmol/g_{wet}) and lactate ($\approx$20 μmol/g_{wet}) contents, one can account for only $\approx$60% of the lactate produced from glycogenolysis during the arrested state. A possible explanation for a discrepancy between measured and calculated lactate production may lie in the formation of lactate from other sources, such as amino acids and/or Krebs cycle metabolites (36). Amino acids can act as metabolic substrates during oxygen insufficiency, using transamination reactions and the malate-aspartate shuttle to form high energy phosphates (37). Pyruvate formed from these reactions can subsequently be converted to lactate via lactate dehydrogenase. Recent studies by Julia *et al.* (38) have shown that such nonglycolytic pathways may become important sources of energy in ischemic puppy hearts.

We have also assessed aerobic metabolism in these neonatal hearts, by measuring rates of glucose oxidation and MV_{O_2}. Under conditions of normal oxygen delivery, rates of glucose oxidation and MV_{O_2} were closely linked to mechanical activity. During low-flow ischemia, the average rate of glucose oxidation decreased to 10% of baseline, consistent with the decrease in oxygen delivery. Nevertheless, glucose oxidation

still provided an important source of ATP during myocardial underperfusion, accounting for $\approx$42% of the net ATP derived from glucose. When indexed to mechanical activity, however, glucose oxidation tended to fall, and glycolysis markedly increased, consistent with the concept of a greater reliance on glycolysis during impaired oxygen availability. Surprisingly, MV_{O_2}, when referenced to mechanical activity, was found to increase ($\approx$40%) during ischemia. A relative increase in oxygen consumption during ischemia would not have been anticipated; however, we have found a similar result with more modest degrees of low-flow ischemia (23), and an increase in oxygen consumption (relative to mechanical activity) has also been reported for globally ischemic immature rabbit hearts (24). In view of the tolerance of neonatal hearts to low-flow ischemia, it is conceivable that this relative increase in oxygen consumption during myocardial underperfusion may result from a beneficial process, *i.e.* an oxygen-consuming process that nevertheless helps maintain ATP stores. Reperfusion was characterized by enhanced glucose oxidation relative to mechanical activity. This finding is consistent with our earlier work which demonstrated an acceleration of fatty acid oxidation in postischemic piglet hearts (23). An additional finding was that glucose oxidation and MV_{O_2} correlated poorly with mechanical activity during reperfusion, indicating a dissociation between contractile function and aerobic metabolism. Such an uncoupling between contractile activity and aerobic metabolism during early reperfusion may reflect an attempt by the heart to replenish ATP stores after the severe ischemic challenge.

Finally, this study implies that myocardial triglycerides undergo hydrolysis during low-flow ischemia. Such a process is of importance, becaue an increase in intracellular FFA could potentially have deleterious effects upon the oxygen limited heart (39, 40). The total glycerol produced, and thus triglyceride hydrolyzed, during ischemia (group I) was $\approx$930 nmol/g_{wet}. The total triglyceride content measured by Werner *et al.* (41) in newborn pig hearts is $\approx$1000 nmol/g_{wet}. Thus, a substantial portion of the myocardial triglyceride pool in our piglet hearts may have undergone lipolysis during the low-flow state. The mechanism underlying an enhanced rate of lipolysis during ischemia is incompletely understood, although Schoonderwoerd *et al.* (16) have provided evidence to suggest that triglyceride breakdown is stimulated by an elevated NADH/NAD$^+$ ratio. Because β-oxidation is impaired during ischemia, re-esterification of the FFA back to triglycerides becomes the primary means of removing the potentially harmful intracellular lipids. This cycle, however, is an ATP-consuming process (16, 42, 43). Based on the glycerol release, and assuming that glycerol 3-phosphate comes from glycogenolysis, we estimate that ATP utilization from the breakdown of triglycerides and complete reesterification of the FFA would constitute only $\approx$6% of the ATP production from glucose.

In summary, we have found that isolated, isovolumically beating neonatal pig hearts develop myocardial contracture when perfused with a crystalloid solution and with a coronary flow reduced to 10% of baseline. Myocardial contracture was associated with a decline in lactate release, suggesting a de-

crease in lactate production and/or impaired lactate clearance. Despite the development of contracture, these hearts exhibited stable rates of glycolysis from exogenously supplied glucose. Moreover, glycolysis accounted for only ≈50% of the lactate produced, which may suggest nonglycolytic sources for energy production. Additionally, glycerol release was enhanced during myocardial underperfusion, indicating hydrolysis of triacylglycerols. Although glucose oxidation was impaired during ischemia, glucose oxidation still provided an important source of ATP. Despite the severe ischemic challenge, these neonatal hearts exhibited functional recovery upon reperfusion. Glycolysis and glucose oxidation returned to baseline values although contractile function was depressed, indicating a dissociation between mechanical and metabolic recovery.

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