High levels of fatty acids increase contractile function of neonatal rabbit hearts during reperfusion following ischemia

Masayoshi Ito,1,4* Jagdip S. Jaswal,1,2* Victoria H. Lam,1 Tatsujiro Oka,1 Liyan Zhang,1,2 Donna L. Beker,1,2 Gary D. Lopaschuk,1,2 and Ivan M. Rebeyka1,3

Mazankowski Alberta Heart Institute, Departments of 1Pediatrics and 2Pharmacology and 3Division of Cardiac Surgery, University of Alberta, Edmonton, Alberta, Canada

Submitted 24 March 2009; accepted in final form 2 February 2010

Ito M, Jaswal JS, Lam VH, Oka T, Zhang L, Beker DL, Lopaschuk GD, Rebeyka IM. High levels of fatty acids increase contractile function of neonatal rabbit hearts during reperfusion following ischemia. Am J Physiol Heart Circ Physiol 298: H1432–H1437, 2010. First published February 12, 2010; doi:10.1152/ajpheart.00284.2009.—In the neonatal heart the transition from using carbohydrates to using fatty acids has not fully matured and oxidative metabolism ATP generation may be limiting contractile function after ischemia. This study tested the hypothesis that increasing fatty acid availability increases recovery of left ventricular (LV) work by increasing palmitate oxidation, tricarboxylic acid (TCA) cycle activity, and ATP generation. Isolated working hearts from 7-day-old rabbits were perfused with Krebs solution containing low (0.4 mM) or high (2.4 mM) palmitate and 5.5 mM glucose. Hearts were subjected to 35-min global ischemia before 40-min reperfusion, and rates of glycolysis, glucose oxidation, and palmitate oxidation were assessed. LV work was similar before ischemia but was greater during reperfusion in hearts perfused with 2.4 mM palmitate compared with hearts perfused with 0.4 mM palmitate [6.98 ± 0.14 (n = 15) vs. 3.01 ± 0.23 (n = 16) mJ·beat−1·g dry wt−1; P < 0.05]. This was accompanied by increased LV energy expenditure during reperfusion [35.98 ± 0.16 (n = 8) vs. 19.92 ± 0.18 (n = 6) mJ·beat−1·g dry wt−1; P < 0.05]. During reperfusion the rates of palmitate oxidation [237.5 ± 28.10 (n = 7) vs. 86.0 ± 9.7 (n = 6) mmol·g dry wt−1·min−1; P < 0.05], total TCA cycle activity [2.65 ± 0.39 (n = 7) vs. 1.36 ± 0.14 (n = 6) mmol acetyl-CoA·g dry wt−1·min−1; P < 0.05], and ATP generation attributable to palmitate oxidation [26.6 ± 3.1 (n = 7) vs. 12.6 ± 1.7 (n = 6) mmol·g dry wt−1·min−1; P < 0.05] were greater in hearts perfused with 2.4 mM palmitate. These data indicate that the neonatal heart has decreased energy reserve, and, in contrast to the mature heart, increasing availability of fatty acid substrate increases energy production and improves recovery of function after ischemia.

fatty acid metabolism; glucose metabolism; cardiac efficiency; cardioprotection

IN PEDIATRIC PATIENTS, corrective surgical procedures in the setting of congenital heart disease(s) require a motionless and blood-free field, necessitating the use of an obligatory period of myocardial ischemia. A marked increase in circulating free fatty acid levels is a hallmark that accompanies myocardial ischemia and reperfusion, and fatty acid levels can remain elevated for upwards of 24 h in pediatric patients (46); however, the effects on the recovery of cardiac function during reperfusion are not yet clearly established. The recovery of cardiac mechanical function after surgical repair is dependent not only on optimal intraoperative cardioprotection but also on cardioprotective interventions utilized during reperfusion as well as the perioperative period. Despite improvements in surgical techniques and procedures utilized in the management of pediatric patients with congenital heart diseases (1, 2, 4, 5, 18, 24), refinements to limit the consequences of ischemia and improve the recovery of postischemic function are necessary, as inadequate cardioprotection remains a major contributor to mortality following surgical repair (3, 4). Activation of prosurvival kinases including Akt and p42-/p44-ERK has emerged as a novel mediator of cardioprotection in response to a variety of interventions (27); however, the activation of these kinases in the neonatal heart remains poorly characterized. Although fatty acids (palmitate) impair the activation of myocardial Akt (64), little is known with regard to the effects of circulating levels of fatty acids on the activation of these prosurvival kinases during reperfusion following ischemia in the neonatal heart.

Under normal, aerobic conditions the mature/adult heart derives 60–70% of its ATP requirements from fatty acid β-oxidation, whereas the remainder of required ATP is derived from the oxidation of carbohydrates, primarily glucose and lactate (17, 47). Immediately after birth, the newborn (1 day old) rabbit heart derives 44–60% of its ATP requirements from glycolysis, whereas lactate oxidation meets the majority of the remaining ATP demand (49). In these hearts, fatty acid β-oxidation only contributes a small fraction (<15%) of total myocardial ATP production (49), and fatty acids provided as a sole energy substrate fail to sustain contractile function in newborn (1 day old) rabbit hearts (48, 71). The contribution of fatty acid β-oxidation markedly increases to become the major source of energy by 14 days of age, rapidly achieving rates seen in the mature/adult heart (37). The maturational changes in fatty acid β-oxidation coincide with changes in the expression and activity of 5’-AMP-activated protein kinase (AMPK), which can phosphorylate and inhibit the activity of acetyl-CoA carboxylase (ACC). After birth, between 1 and 7 days, an increase in the myocardial expression and activity of AMPK (51) and decrease in ACC activity is paralleled by a decrease in malonyl-CoA content (50). Furthermore, during this period there is an increase in the activity of malonyl-CoA decarboxylase (MCD) (58), the enzyme that degrades malonyl-CoA. The alterations in the expression of both ACC and MCD are also observed in the clinical setting in ventricular biopsy samples obtained from neonatal patients (age range 0.2–10 mo) requiring surgical correction of congenital heart defects (72). Alterations in AMPK, ACC, and MCD decrease the malonyl-CoA-induced inhibition of carnitine palmitoyltransferase I (CPT I), the rate-limiting step of mitochondrial fatty acid metabolism.

* M. Ito and J. S. Jaswal contributed equally to this work.

Address for reprint requests and other correspondence: G. D. Lopaschuk, 423 Heritage Medical Research Centre, Depts. of Pediatrics and Pharmacology, Faculty of Medicine and Dentistry, Univ. of Alberta, Edmonton, AB T6G 2S2, Canada (e-mail: gary.lopaschuk@ualberta.ca).
uptake, and allow for fatty acid β-oxidation to become a more predominant contributor to overall cardiac energetics. The decrease in myocardial malonyl-CoA content may also permit fatty acid concentration to become the major regulator of fatty acid β-oxidation; however, this aspect has yet to be assessed. Furthermore, unlike the newborn (1 day old) heart, fatty acids provided as the sole energy substrate can sustain contractile function in neonatal (7 day old) hearts (48). Unlike the marked increase in fatty acid β-oxidation over this time period, the contribution of glucose oxidation only increases by a small amount and remains low compared with the mature/adult heart until weaning (49). This suggests that the transition from the use of carbohydrates to the use of fatty acids is not complete, and that oxidative metabolism/ATP generation may be limiting for postischemic contractile function in the neonatal heart.

In the adult heart the rates of fatty acid oxidation rapidly recover in the postischemic period in the face of depressed contractile function, while the rates of glucose oxidation remain low (40, 41). Pharmacological agents and strategies that shift the balance of oxidative ATP production from fatty acid β-oxidation toward glucose oxidation (20, 21, 39, 45, 52, 65, 67) increase the recovery of postischemic function and cardiac efficiency in the mature/adult heart (42, 44). However, it is not clear whether this finding can be extrapolated to the immature/neonatal heart, which has low glucose oxidation rates. Similar to its effect in mature/adult hearts (42, 44), dichloroacetate (DCA) increases glucose oxidation in postischemic hearts from immature/neonatal (7 day old) rabbits; however, in contrast, it does not increase the recovery of mechanical function (35, 36). This discrepancy may be attributable to the marked differences in metabolic profile between the mature/adult heart and the immature/neonatal heart (described above). Interestingly, increasing perfusate Ca2+ concentration ([Ca2+]p) (from 1.25 to 2.5 mM) increases the postischemic recovery of left ventricular (LV) function in immature/neonatal (7 day old) rabbit hearts, an effect associated with an increase in both glucose oxidation and fatty acid β-oxidation (36). As such, the beneficial effects of increasing [Ca2+]p may be, at least in part, ascribed to increased ATP generation attributable to an increase in overall substrate oxidation, rather than specifically increasing glucose oxidation (36), in addition to potentially surmounting decreases in myofibrillar Ca2+ sensitivity during reperfusion following ischemia (23).

This study investigated whether limited substrate oxidation impacts the recovery of LV function during reperfusion following ischemia in immature/neonatal (7 day old) rabbit hearts. We tested the hypothesis that increasing the concentration of perfusate fatty acid (palmitate) (a major regulator of fatty acid β-oxidation) to levels seen during surgical repair of congenital defects increases oxidative metabolism and ATP generation, and thus improves the recovery of LV function in immature/neonatal (7 day old) rabbit hearts. Furthermore, as palmitate impairs the activation of myocardial Akt (64), we hypothesized that cardioprotection in response to increased perfusate fatty acid concentration is dissociated from the activation of the survival kinases Akt and p42/p44-ERK. As such, we assessed LV work, LV energy expenditure, cardiac efficiency, and the rates of both glucose and fatty acid β-oxidation in neonatal hearts during aerobic perfusion before and after severe global ischemia, as well as the phosphorylation/activation of Akt and p42/p44-ERK at the end of reperfusion.

**MATERIALS AND METHODS**

**Animals.** All animals received humane care according to the guidelines of the Canadian Council on Animal Care, and the studies were approved by the University of Alberta Health Sciences Animal Welfare Committee.

**Heart perfusions.** Hearts from pentobarbital sodium-anesthetized New Zealand White rabbits of either sex (7 day old, 90–200 g) were excised, the aorta was cannulated, and a perfusion using Krebs-Henseleit solution (37°C, pH 7.4, gassed with 95% O2-5% CO2 mixture) was initiated. Hearts were initially perfused in the Langendorff mode for 10 min, after which they were switched to the working/ejecting mode as described previously (38). The perfusate (recirculating volume of 100 ml) consisted of a modified Krebs-Henseleit solution containing 2.5 mM Ca2+, 5.5 mM glucose, 100 μM insulin, and either 0.4 mM palmitate or 2.4 mM palmitate bound to 3% bovine serum albumin. Spontaneously beating hearts were aerobically perfused at a constant workload (preload 7.5 mmHg, afterload 35 mmHg) for a period of 30 min followed by a 35-min period of global, no-flow ischemia and subsequent aerobic reperfusion for 40 min (Fig. 1). Heart rate (beats/min) and systolic and diastolic pressures were measured with a Gould P21 pressure transducer attached to the aortic outflow line. Cardiac output (CO, ml/min) and aortic flow (AF, ml/min) were measured by using ultrasonic flow probes (Transonic T206) placed in the left atrial inflow line and the aortic outflow line, respectively. LV work was calculated as millijoules per beat and normalized to the dry weight of each heart and served as a continuous index of LV mechanical function.

**Measurement and calculation of left ventricular energy expenditure.** Myocardial oxygen consumption (MV02) was calculated according to the Fick principle, using coronary flow [CF (CO – AF)] rates and the perfusate arteriovenous difference in the partial pressure of O2. Arterial and venous gases were assessed with a Radiometer blood gas apparatus (model ABL3m, Radiometer, Copenhagen, Denmark) from samples taken from the left atrial inflow line and a line originating from the cannulated pulmonary artery, respectively. MV02 was calculated as follows:

![Baseline Ischemia Reperfusion](image)

**Fig. 1.** Experimental protocol for heart perfusions. After a 10-min period of Langendorff perfusion, hearts were perfused under aerobic conditions in the working mode for 30 min and subsequently subjected to a 35-min period of global no-flow ischemia, followed by 40 min of aerobic reperfusion in the working mode. Hearts were perfused with either 0.4 mM or 2.4 mM palmitate bound to 3% albumin during the periods of working mode perfusion.
MV0 2 (μmol·g dry wt−1·min−1) = (Pao2 – PVO2) · (CF) · (αo2) · (10^1.5)/dry wt (g) · (P aun − P H2O) · (VO2)  

where PaO2 is the partial pressure of oxygen in the left atrial inflow line, PVO2 is the partial pressure of oxygen in the line originating from the cannulated pulmonary artery, αO2 is the solubility of oxygen (0.0212 ml/ml plasma), P aun is atmospheric pressure (760 mmHg), P H2O is the partial pressure of water (47.1 mmHg), and VO2 is the volume occupied by 1 mol O2 (25.2 l/mol). LV energy expenditure was calculated by converting these values to millijoules per beat normalized to the dry weight of each heart.

Immunoblotting. Frozen ventricular tissue was homogenized in a solution containing (in mM) 20 Tris·HCl (pH 7.4 at 4°C), 50 NaCl, 50 NaF, 5 Na pyrophosphate, 0.25 sucrose, and 1 dithiothreitol, with protease inhibitor cocktail (Sigma) and phosphatase inhibitor cocktail (Sigma). After homogenization for 30 s, protein contents of the homogenates were determined by the Bradford protein assay. Samples were diluted and boiled in protein sample buffer, subjected to SDS-polyacrylamide gel electrophoresis, and transferred to nitrocellulose. Membranes were blocked in 5% (wt/vol) skim milk powder in phosphate-buffered saline (PBS) containing 0.1% (vol/vol) Tween 20 and then immunoblotted with rabbit anti-phospho-Akt (Ser473) (Cell Signaling Technology), anti-Akt (Cell Signaling Technology), anti-phospho-p44/42-ERK (Thr202/Tyr204) (Cell Signaling Technology), anti-p44/42-ERK (Cell Signaling Technology), anti-phospho-AMPK (Thr172) (Cell Signaling Technology), anti-AMPK (Cell Signaling Technology), anti-phospho-ACC (Ser79) (Upstate Cell Signaling Solutions), or peroxidase-labeled streptavidin (Jackson ImmunoResearch Laboratories). After extensive washing, the membranes were incubated with a peroxidase-conjugated goat anti-rabbit secondary antibody in 5% (wt/vol) skim milk powder in PBS when appropriate. After further washing, the antibodies were visualized with an enhanced chemiluminescence Western blotting and detection system (PerkinElmer). Densitometric analyses of immunoblots (n = 7 per experimental group) were performed with Quantity One (4.4.0) Software (Bio-Rad Laboratories). Densitometric values of the phosphorylated proteins were normalized to the total amount of the protein detected and expressed as arbitrary density units (ADU).

Measurement of steady-state rates of glycolysis and glucose oxidation. Glycolysis and glucose oxidation rates were measured directly from the simultaneous production of [3H2O (liberated at the endolase step of glycolysis) and [14CO2 (liberated at the level of enolase step of glycolysis) and 14CO2 (liberated at the level of citric acid cycle), respectively (36). Perfusate was sampled at predetermined time points (10, 20, 30, 64, 70, 80, 90, 100, and 105 min), and steady-state rates (μmol [3H]glucose or [U-14C]glucose metabolized g dry wt−1·min−1) were calculated.

Calculation of rate of proton production arising from metabolism of exogenous glucose. When glucose (from endogenous or exogenous sources) is metabolized by glycolysis and subsequently oxidized 1:1, with the associated synthesis and hydrolysis of ATP, the net production of protons is zero. However, if the rate of glycolysis exceeds that of glucose oxidation, there is a net production of two protons per molecule of exogenous glucose that passes through glycolysis, which is not subsequently oxidized. Therefore, the rate of proton production attributable to the hydrolysis of ATP arising from exogenous glucose metabolism can be calculated as 2·(rate of glycolysis – rate of glucose oxidation).

Measurement of steady-state rates of palmitate oxidation. Steady-state rates of palmitate oxidation were measured from the release of 3H2O derived from the metabolism of [9,10-3H]palmitate. 3H2O was separated from [9,10-3H]palmitate by mixing 0.5 ml of perfusate sampled at predetermined time points (10, 20, 30, 64, 70, 80, 90, 100, and 105 min) with 1.88 ml of a 1:2 (vol/vol) chloroform-methanol solution. Next, 1.0 ml of chloroform was added, followed by the addition of 1.0 ml of KCl·HCl (1.1 mol/l KCl:0.9 mol/l HCl). Samples were allowed to separate into polar and nonpolar phases. A 1.0 ml sample of the polar layer was removed and mixed with 1.0 ml of chloroform, 1.0 ml of methanol, and 0.9 ml of KCl·HCl solution. Again samples were allowed to separate into polar and nonpolar phases, and 0.5-ml aliquots of the polar phase were subjected to scintillation counting for determination of [3H]O2 content. This yielded a >99% separation efficiency of [3H2O from the [3H]palmitate. [3H2O content of the perfusate, indicative of the metabolism of [9,10-3H]palmitate, was determined in samples removed from the perfusate (10, 20, 30, 64, 70, 80, 90, 100, and 105 min) and used to calculate steady-state rates (μmol [9,10-3H]palmitate metabolized g dry wt−1·min−1).

Measurement of malonyl-CoA decarboxylase activity. The activity of MCD was measured as described previously (19). Briefly, ventricular homogenates were incubated in a 210-μl reaction mixture containing 0.1 M Tris, pH 8.0, 0.5 mM dithiothreitol, 50 mM NaF, 5 mM NaPi, and 1 mM malonyl-CoA for 10 min at 37°C. The reaction was stopped by the addition of 40 μl of 0.5 mM perchloric acid, neutralized with 40 μl of 2.2 M KHCO3, (pH 10) and centrifuged at 10,000 g for 5 min to remove precipitated proteins. Incubation of the sample with malonyl-CoA allowed for the conversion of malonyl-CoA to acetyl-CoA, which was then combined with [14C]oxaloacetate (0.17 μCi/ml) to produce [14C]citrate. All reactions were carried out in the presence of N-ethylmaleimide, which removes excess CoA remaining in the latter stages of the reaction so that the citrate present cannot generate non-MCD-derived acetyl-CoA. Unreacted [14C]oxaloacetate was removed from the reaction mixture by the addition of 6.8 mM sodium glutamate and 0.533 μM U/μl aspartate aminotransferase, followed by a 20-min incubation at room temperature. This allowed for transamination of [14C]oxaloacetate back to [14C]aspartate. The resulting solution was then stirred in a 1:2 suspension of Dowex AG 50W-8X resin (100–200 mesh) and centrifuged at 400 g for 10 min. The pelleted Dowex fraction removed [14C]aspartate, while the supernatant fraction contained [14C]citrate. The amount of [14C]citrate present in the form of [14C]citrate in the supernatant fraction was then determined by scintillation counting. The amount of acetyl-CoA produced by MCD was then quantified by comparison to acetyl-CoA standard curves that had been subjected to assay conditions identical to those described above.

Calculation of tricarboxylic acid cycle activity and ATP production rates from rates of energy substrate metabolism. Tricarboxylic acid (TCA) cycle activity was calculated from the rate of acetyl-CoA production attributable to the oxidation of glucose and palmitate. Values of 2 and 8 mol acetyl-CoA/mol of glucose or palmitate oxidized, respectively, were used. The rates of ATP production from glycolysis, glucose oxidation, and palmitate oxidation were calculated with the values of 2 mol ATP/mol glucose passing through glycolysis, 31 mol ATP/mol glucose oxidized, and 105 mol ATP/mol palmitate oxidized.

Measurement of β-hydroxyacyl-CoA dehydrogenase and citrate synthase activities. Enzyme activities were determined as described previously (34, 68). Briefly, frozen ventricular tissue was homogenized in a solution containing (in mM) 20 Tris·HCl (pH 7.4 at 4°C), 50 NaCl, 50 NaF, 5 Na pyrophosphate, 0.25 sucrose, and 1 dithiothreitol, with protease inhibitor cocktail (Sigma) and phosphatase inhibitor cocktail (Sigma). After homogenization for 30 s, protein contents of the homogenates were determined with the Bradford protein assay and Bradford protein assay. Enzyme activities were determined at 30°C for β-hydroxyacyl-CoA dehydrogenase (β-HAD) and at 25°C for citrate synthase with a Spectra Max 190 spectrophotometer (Molecular Devices). For β-HAD activity 15 μg of protein was incubated in a reaction mixture containing (in mM) 50 imidazole (pH = 7.4), 0.15 NADH, and 0.1 acetoacetyl-CoA (omitted for control). β-HAD activity was measured at 340 nm by following the disappearance of NADH (ε = 6.22 μmol·ml−1·cm−1) over 5 min (30-s intervals). For citrate synthase activity 1.2 μg of protein was incubated in a reaction mixture containing (in mM) 100 Tris·HCl (pH = 8.2), 1 MgCl2, 1
EDTA, 0.2 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB) (ε = 13.6 μmol·ml⁻¹·cm⁻¹), 0.3 acetyl-CoA, and 0.5 oxaloacetate (omitted for control). Citrate synthase activity was measured by assessing the rate of change in absorbance at 412 nm over 2 min (30-s intervals).

**Materials.** Radiolabeled substrates ([³H]glucose, [¹⁴C]glucose, and [9,10-³H]palmitate) were purchased from ICN Radiochemicals. Hya-mine hydroxide (methylbenzethonium 1 mol/l in methanol) was purchased from ICN Pharmaceuticals. All other chemicals were reagent grade.

**Statistical analysis.** All values are presented as means ± SE. The significance of differences between two groups was estimated by two-tailed, unpaired Student’s t-test. The significance of differences for multiple comparisons was estimated by one-way analysis of variance (ANOVA). When ANOVA revealed differences, selected data sets were compared by Bonferroni’s multiple comparison test. Differences were considered significant when P < 0.05.

**RESULTS**

Left ventricular work and energy expenditure in neonatal rabbit hearts perfused with palmitate. LV work (mJ·beat⁻¹·g dry wt⁻¹) was similar during the 30-min baseline period of aerobic perfusion between hearts perfused with 0.4 mM palmitate or 2.4 mM palmitate (Fig. 2A). As expected, measurable LV work ceased during the 35-min period of global, no-flow ischemia. Surprisingly, during the period of aerobic reperfusion, the recovery of LV work significantly increased in hearts perfused with 2.4 mM palmitate compared with hearts perfused with 0.4 mM palmitate [6.98 ± 0.14 (n = 15) vs. 3.01 ± 0.23 (n = 16) mJ·beat⁻¹·g dry wt⁻¹; P < 0.05]. Similar effects were observed for the recovery of peak systolic pressure (mmHg), CO (ml/min), and AF (ml/min) during reperfusion as these parameters were increased in hearts perfused with 2.4 mM palmitate compared with hearts perfused with 0.4 mM palmitate (Table 1).

LV energy expenditure (mJ·beat⁻¹·g dry wt⁻¹) was significantly increased in hearts perfused with 2.4 mM palmitate compared with hearts perfused with 0.4 mM palmitate during the baseline period of aerobic perfusion [63.42 ± 0.39 (n = 8) vs. 35.96 ± 0.03 (n = 6); P < 0.05] (Fig. 2B). Since LV work was similar between these groups, cardiac efficiency (measured as LV work/MV̇O₂ and expressed as %) was significantly decreased in hearts perfused with 2.4 mM palmitate compared with hearts perfused with 0.4 mM palmitate [15 ± 0.01% (n = 8) vs. 26 ± 1.6% (n = 6); P < 0.05] (Fig. 2C) during the initial aerobic perfusion.

During reperfusion following ischemia, LV energy expenditure was significantly depressed in both groups of hearts, recovering to ~50% of the values observed during baseline perfusion in both groups of hearts. However, LV energy expenditure (mJ·beat⁻¹·g dry wt⁻¹) was significantly higher in hearts perfused with 2.4 mM palmitate with hearts perfused with 0.4 mM palmitate [35.58 ± 0.16 (n = 8) vs. 19.92 ± 0.18 (n = 6); P < 0.05] during reperfusion following ischemia. Interestingly, cardiac efficiency (%) was also increased in hearts perfused with 2.4 mM palmitate compared with 0.4 mM palmitate during reperfusion following ischemia [20 ± 0.3 (n = 8) vs. 15 ± 1.4 (n = 6)]. These results suggest that increased substrate (palmitate) provision drives oxidative metabolism and the recovery of function in the absence of an oxygen wasting effect during reperfusion following ischemia in hearts from neonatal (7 day old) rabbits.

Phosphorylation of Akt and ERK at end of reperfusion following ischemia. Because palmitate influences the phosphorylation state of myocardial Akt (64), and because the phosphorylation of both Akt and ERK limits postischemic cardiac dysfunction (reviewed in Ref. 28), the phosphorylation of these kinases was assessed in hearts perfused with either 2.4 mM or 0.4 mM palmitate. Despite the marked improvement in the recovery of function during reperfusion following ischemia in hearts perfused with 2.4 mM palmitate, there were no differences in the extent of phosphorylation of Akt (Fig. 3A) or the p44- and p42-ERK isoforms (Fig. 3B) at the end of reperfusion compared with hearts perfused with 0.4 mM palmitate.
Glucose and palmitate metabolism in neonatal rabbit hearts during baseline and reperfusion following ischemia. The rates of glycolysis (\(\mu\text{mol} \cdot \text{g dry wt}^{-1} \cdot \text{min}^{-1}\)) were similar between hearts perfused with 2.4 mM and 0.4 mM palmitate during the baseline period of perfusion [0.75 ± 0.18 (n = 6) vs. 1.07 ± 0.12 (n = 9)] (Fig. 4A). The rates of glycolysis were significantly greater (1.6-fold) during reperfusion compared with baseline in hearts perfused with 0.4 mM palmitate. However, the rates of glycolysis did not differ from those observed during reperfusion in hearts perfused with 2.4 mM palmitate. The rates of glucose oxidation (\(\mu\text{mol} \cdot \text{g dry wt}^{-1} \cdot \text{min}^{-1}\)) were also similar between hearts perfused with 2.4 mM and 0.4 mM palmitate during the baseline period of perfusion [83.51 ± 14.27 (n = 6) vs. 110.79 ± 15.84 (n = 9)] (Fig. 4B). Similar to the rates of glycolysis, the rates of glucose oxidation were greater (1.9-fold) during reperfusion compared with baseline in hearts perfused with 0.4 mM palmitate. However, the rates of glucose oxidation did not differ from those observed during reperfusion in hearts perfused with 2.4 mM palmitate. The rates of proton production (\(\mu\text{mol} \cdot \text{g dry wt}^{-1} \cdot \text{min}^{-1}\)) did not differ between hearts perfused with 0.4 mM palmitate and 2.4 mM palmitate during either baseline or reperfusion (Fig. 4C).

The most profound difference with regard to energy substrate metabolism between the two groups of hearts was seen with palmitate oxidation rates. The rates of palmitate oxidation (\(\mu\text{mol} \cdot \text{g dry wt}^{-1} \cdot \text{min}^{-1}\)) were significantly greater in hearts perfused with 2.4 mM palmitate compared with hearts perfused with 0.4 mM palmitate during the baseline period of perfusion [545.50 ± 62.90 (n = 7) vs. 188.17 ± 15.10 (n = 6); \(P < 0.05\)] (Fig. 4D). During reperfusion, the rates of palmitate oxidation were significantly greater in hearts perfused with 2.4 mM palmitate compared with hearts perfused with 0.4 mM palmitate [237.50 ± 27.10 (n = 7) vs. 86.00 ± 9.70 (n = 6); \(P < 0.05\)].

Phosphorylation of AMPK and ACC at end of reperfusion following ischemia. Because the rates of palmitate oxidation were significantly greater in hearts perfused with 2.4 mM palmitate compared with hearts perfused with 0.4 mM palmitate, differences in the phosphorylation state of both AMPK and ACC between the two groups of hearts were assessed at the end of reperfusion. Despite the elevated rates of palmitate oxidation during reperfusion following ischemia in hearts perfused with 2.4 mM palmitate, there was no difference in the phosphorylation/activation of AMPK compared with hearts perfused with 0.4 mM palmitate at the end of reperfusion (Fig. 5A). Consistent with the lack of alterations in the phosphorylation of AMPK, there was also no difference in the phosphorylation of either ACC\(\beta\) or ACC\(\alpha\) between the two groups of hearts at the end of reperfusion (Fig. 5B). Furthermore, the activity of MCD at the end of reperfusion was similar between hearts perfused with 2.4 mM and 0.4 mM palmitate (Table 2). Taken together, these data indicate that the differences in palmitate oxidation between the two groups of hearts occurred independently of alterations in malonyl-CoA content.
Tricarboxylic acid cycle activity in neonatal rabbit hearts during baseline and reperfusion following ischemia. The production of acetyl-CoA for the TCA cycle, an index of oxidative metabolism, was calculated from the rates of glucose and palmitate oxidation in hearts perfused with either 0.4 mM or 2.4 mM palmitate. In contrast to what is seen in the mature heart (where ~50% of the acetyl-CoA can originate from glucose oxidation), the rates of acetyl-CoA production from glucose oxidation compared with overall rates of acetyl-CoA production were very low in neonatal hearts perfused with 0.4 mM palmitate. In the initial aerobic period, there was very little difference in the contribution of glucose oxidation, regardless of

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Fig. 4. Rates of glucose metabolism, calculated proton production, and palmitate oxidation in hearts perfused with 0.4 mM or 2.4 mM palmitate were assessed as described in MATERIALS AND METHODS. Open bars, hearts perfused with 0.4 mM palmitate; filled bars, hearts perfused with 2.4 mM palmitate. Rates of glycolysis, glucose oxidation, and calculated proton production were determined in hearts perfused with [3H]glucose/[14C]glucose. Rates of palmitate oxidation were determined in hearts perfused with [3H]palmitate/[14C]glucose. Glycolysis (0.4 mM n = 9, 2.4 mM n = 6; A), glucose oxidation (0.4 mM n = 9, 2.4 mM n = 6; B), calculated proton production (0.4 mM n = 9, 2.4 mM n = 6; C), and palmitate oxidation (0.4 mM n = 9, 2.4 mM n = 7; D) during baseline and reperfusion following ischemia are shown. Values represent means ± SE. *Significant difference from baseline within each group; †significant difference between groups during either baseline or reperfusion.

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Fig. 5. Phosphorylation of 5′-AMP-activated protein kinase (AMPK) and acetyl-CoA carboxylase (ACC) in hearts perfused with 0.4 mM or 2.4 mM palmitate was assessed at the end of reperfusion following ischemia as described in MATERIALS AND METHODS. Open bars, hearts perfused with 0.4 mM palmitate; filled bars, hearts perfused with 2.4 mM palmitate. A, top: representative immunoblot of phosphorylated AMPK and total AMPK. B, top: representative immunoblot of phosphorylated ACCα and ACCβ and total ACCα and ACCβ. Bottom: densitometric analysis (0.4 mM n = 7, 2.4 mM n = 7). A, top: representative immunoblot of phosphorylated ACCα and ACCβ and total ACCα and ACCβ. Bottom: densitometric analysis (0.4 mM n = 7, 2.4 mM n = 7).
Table 2. Cardiac malonyl-CoA decarboxylase activity at end of reperfusion following ischemia in neonatal rabbit hearts perfused with palmitate

<table>
<thead>
<tr>
<th>Activity</th>
<th>0.4 mM Palmitate</th>
<th>2.4 mM Palmitate</th>
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<tr>
<td>MCD activity (end reperfusion)</td>
<td>434 ± 51 (7)</td>
<td>588 ± 79 (7)</td>
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Values (in nmol·g dry wt⁻¹·min⁻¹) represent means ± SE for numbers of hearts in parentheses. Malonyl-CoA decarboxylase (MCD) activity at the end of reperfusion following ischemia was calculated in hearts from neonatal (7 day old) rabbits perfused with either 0.4 mM or 2.4 mM palmitate.

whether hearts were perfused with 0.4 mM or 2.4 mM palmitate. In contrast, the rates of acetyl-CoA production from palmitate oxidation were significantly increased [3.94 ± 0.48 (n = 7) vs. 2.10 ± 0.21 (n = 6); P < 0.05] in hearts perfused with 2.4 mM palmitate compared with 0.4 mM palmitate (Fig. 6A).

The rates of acetyl-CoA production from glucose oxidation were similar between the two groups of hearts during reperfusion (Fig. 6B); however, the rates of acetyl-CoA production attributed to palmitate oxidation were significantly greater in hearts perfused with 2.4 mM palmitate compared with hearts perfused with 0.4 mM palmitate [2.36 ± 0.40 (n = 7) vs. 0.96 ± 0.13 (n = 6); P < 0.05] (Fig. 6B). As palmitate oxidation was the major contributor to acetyl-CoA production, the total rates of acetyl-CoA production were also greater in hearts perfused with 2.4 mM palmitate compared with hearts perfused with 0.4 mM palmitate (Fig. 6A).

Activity of β-hydroxyacyl-CoA dehydrogenase and citrate synthase in rabbit hearts at end of reperfusion following ischemia. The activities of β-HAD and citrate synthase were measured to determine whether differences in the capacity to utilize palmitate via fatty acid β-oxidation or the capacity of the TCA cycle, respectively, account for the differences in calculated acetyl-CoA production arising from palmitate oxidation during reperfusion. The activities of β-HAD (Fig. 7A) and citrate synthase (Fig. 7B) did not differ at the end of reperfusion, suggesting that the capacity to utilize palmitate via β-oxidation and the capacity of the TCA cycle are similar between hearts perfused with 2.4 mM and 0.4 mM palmitate. These data suggest that fatty acid provision may be limiting oxidative metabolism in the posts ischemic neonatal heart. To examine this directly, an additional series of hearts were perfused in which fatty acid concentration was increased from 0.4 mM palmitate to 2.4 mM palmitate during both the baseline and reperfusion periods. Increasing fatty acid concentration during baseline from 0.4 mM palmitate to 2.4 mM palmitate did not alter LV work. However, increasing fatty acid concentration during reperfusion increased the recovery of posts ischemic LV work as a percentage of baseline LV work where hearts were perfused with 0.4 mM palmitate compared with the
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Table 3. Recovery of postischemic function in neonatal rabbit hearts when perfusate fatty acid concentration is increased from 0.4 mM (baseline) to 2.4 mM (reperfusion) palmitate or decreased from 2.4 mM (baseline) to 0.4 mM (reperfusion) palmitate

<table>
<thead>
<tr>
<th>Source of ATP</th>
<th>Baseline</th>
<th>Reperfusion</th>
<th>Recovery, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycolysis</td>
<td>2.2 ± 0.3 (9)</td>
<td>1.3 ± 0.4 (6)</td>
<td>3.6 ± 0.4 (9)*</td>
</tr>
<tr>
<td>Glucose oxidation</td>
<td>3.7 ± 0.3 (10)</td>
<td>2.8 ± 0.4 (9)</td>
<td>5.8 ± 0.6 (10)*</td>
</tr>
<tr>
<td>Palmitate oxidation</td>
<td>19.8 ± 1.6 (6)</td>
<td>57.3 ± 6.6 (7)†</td>
<td>8.9 ± 1.0 (6)*</td>
</tr>
</tbody>
</table>

Values (in mJ·beat⁻¹·g dry wt⁻¹) represent means ± SE. Left ventricular (LV) work was assessed in neonatal (7 day old) rabbit hearts during both baseline and reperfusion, when perfusate fatty acid concentration was varied from 0.4 mM and 2.4 mM palmitate during each phase of the perfusion protocol (n = 18). In series 1 recovery (%) represents reperfusion with 2.4 mM palmitate vs. baseline perfusion with 0.4 mM palmitate, whereas in series 2 recovery (%) represents reperfusion with 0.4 mM palmitate vs. baseline perfusion with 2.4 mM palmitate. *Significant difference between series during either baseline or reperfusion.

DISCUSSION

This study investigated the effects of increasing energy substrate supply, specifically fatty acid provision, on the recovery of cardiac function during reperfusion following ischemia in the neonatal (7 day old) rabbit heart. Neonatal rabbit hearts were perfused with levels of fatty acids normally seen under aerobic conditions (0.4 mM palmitate) as well as the levels of fatty acids that can be seen in the newborn/neonate after cardiac surgery (46). A number of important and novel findings emerged from this study. First, supporting previous studies, we demonstrate that glucose oxidation is a minor contributor to TCA cycle activity and energy production in hearts perfused with normal levels of fatty acid (0.4 mM palmitate), which confirms the delayed maturation of glucose oxidation seen in the neonatal heart. Of importance is the finding that increasing fatty acid concentration from 0.4 mM to 2.4 mM palmitate had very little effect on glucose oxidation but did markedly increase overall energy supply from fatty acid β-oxidation. Surprisingly, unlike the mature heart, we demonstrate that increasing fatty acid concentration from 0.4 mM to 2.4 mM increased the recovery of cardiac function after global ischemia. This effect was accompanied by increased LV energy expenditure and an increase in the rates of palmitate oxidation during reperfusion. As such, during reperfusion TCA cycle activity and the rates of ATP production attributable to fatty acid β-oxidation were elevated in hearts perfused with 2.4 mM palmitate. These data indicate that, in contrast to the adult heart where increased rates of fatty acid β-oxidation are detrimental to the recovery of cardiac work after ischemia, increasing fatty acid oxidation drives oxidative metabolism and hence ATP generation in the neonatal heart, which exerts salutary effects on the recovery of postischemic function. This has important implications for potential strategies used to optimize energy metabolism in the neonatal heart after cardiac surgery and suggests that increasing energy metabolism from fatty acid β-oxidation, as opposed to preventing the fatty acid-induced inhibition of glucose oxidation, can benefit the neonatal heart after ischemia.

Previous reports utilizing the open-chest, instrumented rabbit heart model demonstrate that coronary perfusion pressure approximates 64–65 mmHg (59, 60) in neonatal (<1 wk of age) rabbits in vivo. This study utilized the isolated working/
ejecting neonatal rabbit heart preparation, a well-characterized model for the simultaneous assessment of cardiac function and metabolism. In the preparation utilized in this study coronary perfusion pressure is set by afterload, equivalent to 35 mmHg. Clearly this is less than that observed in vivo. However, previous reports have characterized the experimental model utilized in this study, demonstrating its ability to respond to increases in afterload from 20 mmHg to 30 mmHg with increases in peak systolic pressure and mechanical function, whereas this preparation in unable to eject perfusate against an afterload ≥40 mmHg (48). As such, these differences in coronary perfusion pressure may have influenced the findings of this study. In addition, the energy substrates supplied to the isolated working heart may not accurately reflect the milieu of substrates to which the heart is exposed to in vivo. This is also extrapolated to a lack of other circulating factors (e.g., hormones) that are present in vivo. However, the ability to control the energy substrates to which the isolated heart is exposed to allows characterization of the interaction between the selected energy substrates (6).

Hearts were perfused with glucose and palmitate as energy substrates, and as such oxidative metabolism is only attributable to these two energy substrates. Therefore, the findings of this study may be superimposed upon possible limitations in carbon substrates provided to the neonatal heart. Interestingly, although lactate represents an important energy substrate in the fetal (7) and immediate newborn (2-day-old pig hearts) (70), although lactate represents an important energy substrate in the fetal (7) and immediate newborn (2-day-old pig hearts) (70), this study may be superimposed upon possible limitations in coronary perfusion pressure may have influenced the findings of this study. In addition, the energy substrates supplied to the isolated working heart may not accurately reflect the milieu of substrates to which the heart is exposed to in vivo. This is also extrapolated to a lack of other circulating factors (e.g., hormones) that are present in vivo. However, the ability to control the energy substrates to which the isolated heart is exposed to allows characterization of the interaction between the selected energy substrates (6).

LV energy expenditure, as expected (33, 69), was increased in hearts perfused with 2.4 mM palmitate both during the baseline period of perfusion and during reperfusion. Interestingly, during the initial aerobic perfusion TCA cycle activity and LV energy expenditure were increased in hearts perfused with 2.4 mM palmitate compared with hearts perfused with 0.4 mM, despite similar cardiac function. As a result, cardiac efficiency (i.e., LV work/LV energy expenditure) was actually depressed in hearts perfused with 2.4 mM palmitate compared with hearts perfused with 0.4 mM palmitate. This effect parallels observations in the perfused mouse heart, where decreased cardiac efficiency in response to elevations in fatty acid concentration results from increased MV\(_{\text{O}}\) and not depressed cardiac function (31). Oxygen wasting in the presence of elevated fatty acid levels is at least in part attributed to the increased oxygen cost of ATP generation when using fatty acids versus glucose as fuel. However, this can only account for a 10–12% difference in efficiency when using exclusively glucose versus palmitate as an energy substrate, a value that is low compared with the 35% difference in efficiency observed in this study (29). The remainder of the oxygen wasting effect may be due to mitochondrial uncoupling (12, 14, 15) and/or futile intracellular cycles utilized by fatty acids including the cycling of fatty acids between long-chain acyl-CoA synthase and mitochondrial and cytosolic thioesterase reactions (13), as well as triglyceride-fatty acid cycling (56). However, these parameters were not assessed in this study. Interestingly, during reperfusion an oxygen wasting effect was not evident, as the recovery of LV work, LV energy expenditure, and cardiac efficiency was actually increased in hearts perfused with 2.4 mM palmitate compared with hearts perfused with 0.4 mM palmitate. These effects are contrary to those observed in hearts from adult animals (42–44) and may be related to the unique metabolic profile of the neonatal (7 day old) rabbit heart.

Although cardiac efficiency is commonly assessed by relating external ventricular work (i.e., LV work) to LV energy expenditure, it must be taken into account that this ratio is dependent on workload (30, 66). Therefore, this parameter is valuable for comparing cardiac efficiency between hearts perfused with 0.4 mM palmitate and 2.4 mM palmitate during the baseline period of perfusion, where LV work is equivalent between the two groups. Thus, during reperfusion, where LV work differs between hearts perfused with 0.4 mM palmitate compared with hearts perfused with 2.4 mM palmitate, this ratio must be interpreted with caution. In such a scenario where LV work differs between hearts, the most accurate method of calculating and comparing cardiac efficiency involves the use of the pressure-volume area (PVA)-MV\(_{\text{O}}\) relationship (30). As PVA strongly correlates with MV\(_{\text{O}}\) over a wide range of workloads, it thereby allows the comparison of cardiac efficiency between hearts performing different degrees of external work (30). However, in this study cardiac efficiency cannot be calculated in this manner, as calculation of PVA requires direct measurement of LV pressure whereas the present study measured aortic pressure. Thus it will be important to verify that the reported differences in cardiac efficiency (calculated as LV work/LV energy expenditure) observed during reperfusion do indeed persist when cardiac efficiency is calculated with the workload-independent PVA-MV\(_{\text{O}}\) relationship.

Akt and ERK are important components of the reperfusion injury salvage kinase (RISK) pathway that can be stimulated by insulin and that act to limit infarct size and postischemic mechanical dysfunction (28). Interestingly, although the recovery of mechanical function was greater in hearts perfused with 2.4 mM palmitate, the extent of Akt and p42/p44-ERK phosphorylation did not differ from that in hearts perfused with 0.4 mM palmitate. This is surprising considering the ability of palmitate to impair insulin-stimulated Akt phosphorylation in the adult rat heart (64), a finding that may actually suggest decreased Akt phosphorylation in neonatal (7 day old) rabbit hearts perfused with elevated levels of fatty acids. The reasons for these differences are not known but may be related to potential species differences or differences in myocardial insulin sensitivity between the adult and neonatal heart; however, these facets were not assessed in this study. The increased phosphorylation of Akt and p42/p44-ERK following various cardioprotective interventions is usually documented within the first 5–15 min of reperfusion (22, 26, 32, 61). In this study the phosphorylation of Akt and p42/p44-ERK was assessed
after 40 min of reperfusion, and as such possible temporal changes in Akt and p42/p44-ERK that occur early in reperfusion may have been missed, although there are reports implicating sustained increases in the phosphorylation of Akt and p42/p44-ERK following 2 h of reperfusion in mediating cardioprotection (55).

The rates of glycolysis and glucose oxidation were similar during baseline in hearts perfused with 0.4 mM or 2.4 mM palmitate; however, each of these rates increased during reperfusion in hearts perfused with 0.4 mM palmitate. This suggests that while glucose metabolism has not fully matured in hearts from neonatal (7 day old) rabbits (37, 48, 49), the reciprocal relationship between fatty acid and glucose metabolism (i.e., Randle cycle) is nonetheless present. As glucose metabolism has not fully matured, its capacity to meet the energetic requirements of hearts from neonatal (7 day old) rabbits during reperfusion following ischemia is limited. This observation is supported by previous findings demonstrating that although DCA increases glucose oxidation during reperfusion, it is not sufficient to increase the recovery of cardiac function in hearts from neonatal (7 day old) rabbits (35). In contrast to observations in hearts from adult animals, where high levels of fatty acids markedly uncouple glycolysis and glucose oxidation (38), the rates of proton production were similar in hearts from neonatal (7 day old) rabbits perfused with 0.4 mM and 2.4 mM palmitate, which is likely attributable to the limited capacity of glucose oxidation in these hearts. Furthermore, the lack of effect on proton production between hearts perfused with 0.4 or 2.4 mM palmitate may limit potential disturbances in intracellular Na⁺ and Ca²⁺ homeostasis, which can impact ischemic injury (16, 34).

Fatty acid β-oxidation quickly matures after birth and is the major source of oxidative ATP production compared with glucose oxidation in hearts from 7-day-old rabbits (Table 4; Ref. 49). Increasing fatty acid concentration further increases the rates of palmitate oxidation, suggesting that hearts from neonatal (7 day old) rabbits possess some degree of energetic reserve and can increase fatty acid β-oxidation, an effect accompanied by an increased recovery of cardiac function during reperfusion. These effects are corroborated by previous findings demonstrating that increasing myocardial pyruvate metabolism (63), or increasing perfusate Ca²⁺ concentration, increases the recovery of function after ischemia in hearts from neonatal (7 day old) rabbits. As pyruvate can increase sarcoplasmic reticulum Ca²⁺ content (25, 73), and intracellular Ca²⁺ transients (25), its effects on postischemic function in the neonatal rabbit heart, similar to increasing perfusate [Ca²⁺], may be at least in part attributable to an increase in overall oxidative metabolism (i.e., fatty acid β-oxidation and glucose oxidation) (36) as opposed to stimulating the limited capacity of glucose oxidation per se (35, 36), in addition to surmounting potential decreases in myofibrillar Ca²⁺ sensitivity in the postischemic period (23). This may be particularly relevant in the neonatal heart, which has a greater requirement for extracellular Ca²⁺ for contraction (62) and a weaker state of developed sarcoplasmic reticulum (10, 11). Despite the marked differences in palmitate oxidation between hearts perfused with 2.4 mM and 0.4 mM palmitate, there were no differences in the phosphorylation of either AMPK or ACC or the activity of MCD, suggesting that the observed differences in fatty acid β-oxidation occurred independently of alterations in malonyl-CoA content. Although malonyl-CoA content was not measured in this study, these findings are consistent with previous findings in the newborn heart, where the adiponectin-induced stimulation of palmitate oxidation occurs independently of changes in AMPK and ACC phosphorylation as well as malonyl-CoA content (57). Therefore the elevated rates of palmitate oxidation observed in hearts perfused with 2.4 mM palmitate compared with hearts perfused with 0.4 mM palmitate are likely attributable to increased fatty acid provision, and not differences in the allosteric regulation of fatty acid β-oxidation.

Total TCA cycle activity recovered during reperfusion to similar extents in hearts perfused with either 0.4 mM or 2.4 mM palmitate (58% vs. 65% of baseline values), similar to previously reported values (43). However, total TCA cycle activity in hearts perfused with 0.4 mM was only 51% of that in hearts perfused with 2.4 mM palmitate. This suggests that 1) it is not the relative proportion, but rather absolute TCA cycle activity, that accounts for the differences in functional recovery and 2) increasing fatty acid provision increases fatty acid oxidation, and hence absolute TCA cycle activity. These data are further corroborated by the finding that despite differences in the recovery of postischemic function, the activities of β-HAD (an index of total fatty acid β-oxidative capacity) and citrate synthase (an index of the capacity of the TCA cycle), and hence indexes of oxidative capacity (54, 68), are similar between hearts perfused with 2.4 mM and 0.4 mM palmitate. Thus the increased rates of acetyl-CoA generation attributable to palmitate oxidation during reperfusion may be due to increased palmitate provision in hearts perfused with 2.4 mM palmitate rather than differences in energy demand. The ability of increasing fatty acid provision to increase the recovery of function may also be related to the lack of effect on proton production (described above) and the ability of hearts of neonatal animals to more efficiently utilize MV˙O₂ and therefore ATP for contractile purposes compared with the adult heart, owing possibly to lower wall stress and thus oxygen demand (59). Alternatively, differences in ATP content at the end of the ischemic period between hearts perfused with 0.4 mM palmitate and 2.4 mM palmitate may contribute to the differences in recovery during reperfusion. Interestingly, the rates of ATP production during the baseline period (before ischemia) were greater (2.9-fold) in hearts perfused with 2.4 mM palmitate compared with hearts perfused with 0.4 mM palmitate and may thus suggest that ATP content before entering the ischemic period, as well as at the end of the ischemic period, was greater in these hearts; however, this was not assessed in this study. Furthermore, it should be noted that the calculated rates of ATP production do not take into account the efficiency of ATP synthesis, and that there was some degree of oxygen waste as evidenced by the reduction in cardiac efficiency during the baseline period in hearts perfused with 2.4 mM palmitate, which would reduce the efficiency of ATP synthesis. Therefore, the contribution of differences in ATP content before and immediately after the ischemic period to the observed effects remains unresolved, and requires further characterization.

In conclusion, this study demonstrates that increasing perfusate fatty acid concentration increases the recovery of postischemic cardiac function in hearts from neonatal (7 day old) rabbits. This occurred in the absence of an oxygen wasting effect during reperfusion, as increased TCA cycle activity and hence oxidative ATP generation and subsequent ATP hydro-
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lysis were efficiently converted to contractile function. This indicates that the heart in the neonate possesses an energy reserve, where increasing oxidative metabolism, specifically by increasing fatty acid provision, has salutary effects on cardiac function. Thus metabolic support in the form of increased fatty acid availability may have the potential to limit the deleterious consequences of ischemia in the neonatal heart.

GRANTS

Research funding for this study was provided by a grant from the Canadian Institutes of Health Research to G. D. Lopaschuk and I. M. Rebeyka. G. D. Lopaschuk is an Alberta Heritage Foundation for Medical Research Scientist. V. H. Lam is supported by studentship awards from the Alberta Heritage Foundation for Medical Research and the Canadian Institutes of Health Research.

DISCLOSURES

No conflicts of interest are declared by the author(s).

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