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Myocardial Energy Metabolism in the Newborn Lamb *In Vivo* during Pacing-Induced Changes in Oxygen Consumption

MICHAEL A. PORTMAN AND XUE-HAN NING

Division of Cardiology, Department of Pediatrics, School of Medicine, University of Washington, Seattle, Washington 98195, and Department of Radiology, College of Human Medicine, Michigan State University, East Lansing, Michigan 48824

ABSTRACT

Myocardial energy metabolism was studied in newborn sheep to determine whether the metabolic responses to pacing-induced increases in heart rate were similar to those previously found during catecholamine stimulation. Open-chest newborn sheep, 3 to 9 d old (n = 11), underwent atrial pacing at a respiratory rate harmonic just above the intrinsic heart rate. Pacing rate was increased by 30 beats/min every 5 min until conduction block or a drop in systemic arterial pressure occurred. Phosphorous metabolites were monitored simultaneously (n = 7) using a ^{31}P magnetic resonance surface coil over the heart within a magnet operating at 4.7 tesla. Myocardial oxygen consumption was monitored via an extracorporeal shunt from the coronary sinus. Rate pressure product increased with heart rate and was found to relate to myocardial oxygen consumption (r = 0.75), which increased maximally by 47 ± 9% due to increases in coronary blood flow. Phosphocreatine/ATP ratio decreased significantly, and calculated ADP increased between baseline and peak performance but returned to near baseline levels during recovery at the initial pacing rate. These findings indicate that intracellular high-energy phosphate concentrations do change with alterations in myocardial oxygen consumption induced by cardiac pacing in the newborn. These changes are similar to those found during epinephrine infusion. Furthermore, the ATP hydrolysis products probably participate in myocardial respiratory regulation in the newborn *in vivo*. (***Pediatr Res* 37: 182–188, 1995**)

Abbreviations

MVO_2, myocardial oxygen consumption
PCr, phosphocreatine
NMR, nuclear magnetic resonance
P_i, intracellular phosphate
RPP, rate pressure product
bpm, beats per minute

Major modifications in myocardial energy metabolism and regulation occur shortly after birth (1, 2). ATP production is tightly coupled to utilization in the newborn myocardium as well as the mature myocardium. However, epinephrine-induced increases in cardiac oxygen consumption and work rates in the newborn *in situ* are associated with corresponding changes in ATP hydrolysis products (1, 3). These high-energy phosphate alterations are not evident in the older animal. This finding would seem to indicate that the newborn myocardium, unlike the mature myocardium, uses these products, ADP and P_i, in a feedback or kinetic mode of respiratory control. Increasing cytosolic ADP and P_i would thereby stimulate mitochondrial oxidative phosphorylation. Alternatively, this relation between ADP, P_i, and oxygen consumption in the newborn may reflect developmental modifications in substrate utilization precipitated directly by exogenous catecholamines. NADH/NAD theoretically exists in equilibrium with phosphorylation potential, ATP/ADP·P_i, and is influenced by reducing equivalent delivery or substrate supply (4). Studies performed both in perfused hearts (5) and in intact animals (6) indicate that phosphorylation potential can be altered through changes in substrate utilization without concomitant changes in oxygen consumption. Accordingly, the previous demonstration of lower phosphorylation potential in newborn myocardium (7) is presumably due to increased dependence on carbohydrate metabolism (8, 9) with a resulting lower NADH/NAD ratio. Conceivably, this ratio might also be altered by maturationally related changes in systemic and myocardial substrate availability induced by exogenous epinephrine, either in addition to or exclusive of simultaneous changes in oxygen consumption. The concurrence of oxygen consumption and substrate utilization changes *in vivo* causes difficulty in separation and study of each individual phenomenon and its respective effect on phosphorylation potential. Two approaches may be taken to examine these relationships in the newborn animal *in vivo*. First, substrate utilization must be altered without concordant change

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Correspondence: Michael A. Portman, M.D., Department of Pediatrics RD/20, University of Washington, School of Medicine, Seattle, WA 98195.
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in oxygen consumption. Second, oxygen consumption may be stimulated with minimal direct catecholamine and substrate alterations. In this study, the latter method was applied to increase oxygen consumption, without the concordant changes in substrate supply and utilization typically caused by epinephrine infusion. Atrial pacing in a group of anesthetized newborn lambs was used to induce relatively small but significant changes in MVO_2. This group was similar in age distribution to a population previously used to study high-energy phosphate metabolism during epinephrine-induced oxygen consumption increases. Using this group, we tested the hypothesis, which states that high-energy phosphate levels will remain stable during such oxygen consumption increases, inasmuch as previously reported alterations in newborn lambs were due to a specific epinephrine effect.

METHODS

Animal preparation. Animals used in this study were handled in accordance with institutional and National Institutes of Health animal care and use guidelines. Mixed breed newborn lambs ranging between 3 and 9 d old (mean 6.1 ± 1.2 d, n = 11) and weighing between 4 and 6 kg were used for this study.

Animals were sedated with an intramuscular injection of 10 mg/kg ketamine and 0.2–0.4 mg/kg xylazine and intubated; anesthesia was induced with a mixture of 0.5–1.0% halothane and ~99% oxygen. This was followed with an i.v. dose of α-chloralose (60 mg/kg) and discontinuation of halothane inhalation. Femoral arterial cannulation was performed for monitoring systemic blood pressure and sampling blood. Arterial pH was maintained between 7.35 and 7.45 by adjustment of ventilatory tidal volume and correction of metabolic acidosis with sodium bicarbonate infusion. After a median sternotomy, the pericardial fat pad was exposed and removed. Platinum-tipped pacing electrodes were sutured to the right atrial appendage. Coronary sinus flow was measured as previously described via an extracorporeal shunt between the coronary sinus and the superior vena cava fashioned by cannulating both jugular veins with heparin-flushed Tygon tubing (Tygon, Norton Performance Plastics, Akron, OH; ⅛-inch internal diameter, [3/16]-inch outer diameter). One tube end was advanced retrograde into the coronary sinus, whereas the other was positioned in the superior vena cava. A cannulating ultrasonic transit time probe was inserted in the tubing for continuous measurement of shunt flow, as was a T connector for sampling for oxygen content. The sinus catheter tip was positioned between the coronary sinus orifice and the juncture of the great cardiac and hemizygous veins. A suture was placed around the coronary sinus to anchor the tip and direct all coronary sinus drainage into the Tygon shunt. The hemizygous vein, which drains directly into the coronary sinus in sheep, was then ligated. The flow-monitoring system provided digital display and continuous hard copy tracing. An ellipsoid-shaped NMR surface coil measuring 2 cm in the major axis, and conforming to the general shape of the sheep heart, was sutured to the pericardium overlying the left ventricle (n = 7). This coil placement allowed unrestricted cardiac movement and filling, while keeping the coil in reproducible proximity to the heart. The thoracotomy opening was then sealed with plastic wrap to prevent water loss. The lamb was wrapped in a water-circulating heating blanket that maintained body core temperature at ~38°C and placed in a Lucite cradle that fit into the 26-cm clear bore of the 4.7-tesla chemical shift imager system. The surface coil was positioned at the magnetic center of the system.

NMR measurements. After transfer of the lamb into the magnet, the NMR surface coil was tuned to 81 MHz and matched to 50 ohms using a network analyzer with a connecting cable calibrated for use within the magnet field. Respiration was maintained within the magnet via 15 feet of tubing attached to the expiratory and inspiratory ports of the ventilator. Blood pressure was monitored using a solid-state nonmagnetic pressure transducer (Cobe Lab Inc., Lakewood, CO) positioned just outside the magnet bore.

NMR data were collected with a General Electric (Fremont, CA) spectrometer using OMEGA software. Shimming on the ^1H free induction decay at 200 MHz and acquisition of ^{31}P spectra were performed as previously described (1, 3, 7), using both cardiac and respiratory gating. This was done by using the Labtech Notebook program (Laboratory Technologies Corp., Wilmington, MA) and a personal computer that synchronized cardiac pacing and NMR acquisitions to a harmonic of the respiratory rate (30 breaths/min). NMR spectra were obtained using a one-pulse sequence with a pulse width optimized to maximize PCr signal and a 2-s interpulse delay.

Protocol. The hearts were atrially paced at the lowest respiratory rate harmonic above the intrinsic heart rate, either 120, 150, or 180 bpm to maintain synchrony of heart rate, respiration, and NMR acquisition. Such synchrony is required to optimize the NMR signal to noise ratio and resolution. After 5 min of baseline data acquisition, the heart rate was increased to the next harmonic for a 5-min period. Systemic arterial and coronary venous samples were obtained at 2 and 4 min into the period. Blood pH, Po_2, and Pco_2 were determined using a blood gas analyzer (ABL330, Radiometer, Copenhagen, Denmark). Oxygen content was determined using the ABL330 in conjunction with a hemoximeter (OSM3, Radiometer), which measured sheep Hb saturation and then calculated total oxygen content from dissolved oxygen according to the Po_2 and from the oxyhemoglobin content. This protocol was then repeated at progressively increasing harmonics until either a conduction block appeared or mean aortic pressure decreased 10 mm Hg below the baseline level. Pacing was then restarted at the baseline level for a 5-min recovery period. ^{31}P spectra were obtained throughout the experiment in 1-min (30 acquisitions) blocks. The final 4 min of spectra from each pacing period were summed and used for analysis. After protocol completion, animals were killed with a KCl infusion, the hearts were excised, the right ventricle and atria were removed, and the left ventricle was weighed.

Substrate uptake. FFA, lactate, and glucose uptake were measured in a separate group of lambs (n = 4) with similar age and weight distribution that underwent the identical pacing protocol. NMR studies were not performed in this group. Simultaneous arterial and coronary sinus samples were drawn for substrate analysis and oxygen content during each pacing

increment. The heparinized sample was then centrifuged and the serum frozen. Lactates and glucose were measured using a GM7 analyzer (Analox Instruments, Optimetrix Inc., Ayer, MA).

Catecholamine levels. Fractionated and serum catecholamine levels were measured in three additional lambs with similar age distribution using the pacing protocol. After the pacing protocol, the heart rate was returned to baseline and epinephrine 1–2 $\mu g \cdot kg^{-1} \cdot min^{-1}$, sufficient to raise coronary blood flow rate by 50%, was infused for 10 min. During baseline and at peak pacing rates, as well as steady state during epinephrine infusion, 5 mL of heparinized arterial blood were collected into a tube containing 5 mg of solid sodium metabisulfite, which serves as an antioxidant. The tubes were immersed immediately in ice and centrifuged at 2–8°C. Samples were immediately frozen at less than −70°C. Samples were then analyzed at the University of Washington Medical Center, Department of Laboratory Medicine. Standard HPLC techniques were used with a silica-based cation exchange column, which separates the fractions epinephrine, norepinephrine, dopamine, and internal standard isocratically (Bio-Rad Instruction Manual no. 195-6051, Plasma Catecholamines by HPLC, Bio-Rad Laboratories, Hercules, CA).

Data analysis. All peak areas of PCr and β-ATP were determined using a deconvolution line-fitting program and integration. Ratios were corrected for saturation effects by comparison with fully relaxed spectra. The concentration of ADP was calculated from the creatine kinase equilibrium reaction (brackets indicate "concentration"): $[ADP] = [ATP][Cr]/K_{eq}[PCr][H^+]$, where the equilibrium constant, K_{eq}, is 1.66×10^9 as reported by Lawson and Veech (10). Published freeze extraction values of 8.6 mM for ATP and 20.4 mM for total creatine were used for these calculations (1). In sheep blood, 2,3 diphosphoglycerate occurs at levels too low to significantly contaminate the inorganic phosphate peak (7). Therefore, intracellular pH was calculated from the chemical shift of the P_i peak referenced to PCr. MVO_2 was determined from coronary sinus flow × coronary arterial-venous oxygen content difference normalized for left ventricular wet weight and expressed as μmol/g/min.

Statistical analysis. Parameters at baseline and at peak level of oxygen consumption were compared using a two-tailed paired *t* test. Similarly, recovery was compared with baseline. Linear regression analysis was used to determine whether a relationship between various parameters at multiple heart rates steps existed. Slopes of lines were compared with slope = 0 using a *t* test. All descriptive statistical data are reported as means ± SEM. $p < 0.05$ was considered significant in all statistical tests.

RESULTS

Table 1 summarizes the important hemodynamic and metabolic parameters obtained from these experiments.

Hemodynamic variables. The pacing rate was always a harmonic of the respiratory rate (30 breaths per min), thus yielding an initial rate of either 150 or 180 in these newborn lambs. In two animals, the intrinsic heart rate had risen to a level higher than the baseline pacing rate so that the recovery period heart rate was higher than baseline. There was no significant change in mean arterial pressure when comparing baseline with peak heart rate or recovery periods. Figure 1 illustrates a continuous recording of simultaneous systemic arterial pressure and coronary sinus flow. Systolic/diastolic blood pressure is intermingled with the mean tracings. Breaks in the tracings occur during periods of blood sampling. Changes in heart rate are noted in Figure 1. In this particular experiment, heart rate is increased by the harmonic (30 bpm) for 5-min periods until there is a drop in mean arterial blood pressure and coronary sinus flow at 330 bpm. Peak RPP and oxygen consumption data for this experiment were extracted at 300 bpm.

RPP and oxygen consumption. Mean RPP increased substantially between baseline and peak performance. The individual animal data are plotted in Figure 2. The number of pacing rate increments varied with the individual animal from two to five, as did the maximal tolerated heart rate. This variability precluded the use of many statistical techniques that require fairly rigid protocols. Linear regression analysis was therefore used to determine whether a relationship existed between oxygen consumption and RPP. This yielded the regression equation $RPP = 6367MVO_2 + 2047$ ($r = 0.75$). Furthermore, *t* test comparison revealed that the slope of this line was significantly different from 0, indicating that RPP correlates with MVO_2. Additionally, peak RPP and MVO_2 were significantly higher than baseline, but baseline values were not statistically different from recovery values. Oxygen consumption changed by 47 ± 9% from baseline to peak periods. This was due primarily to increases in coronary flow, inasmuch as the coronary oxygen extraction [(arterial − coronary sinus oxygen content)/arterial oxygen content] did not change significantly during these pacing protocols.

Table 1. *Hemodynamic and metabolic effects of increasing HR in seven newborn sheep*

Period	HR	BP	RPP	MVO_2	CSFlow	PCr/ATP
Baseline	167 ± 7	66 ± 3	11 047 ± 765	3.2 ± 0.56	0.78 ± 0.09	1.55 ± 0.10
Peak	263 ± 20*	61 ± 3	15 937 ± 1 475*	4.7 ± 0.65†	1.01± 0.15†	1.36 ± 0.07*
Recovery	170 ± 6	65 ± 5	11 0.25 ± 1 132	3.67 ± 0.68	0.94 ± 0.15	1.67 ± 0.17

Baseline is at the initial pacing rate. Peak is defined as pacing period where maximum oxygen consumption rate or maximum RPP was achieved. Recovery is recovery period after return to initial heart rate. HR is in bpm; BP is mean arterial pressure in mm Hg; RPP is HR·BP; MVO_2 is in μmol/g/min; CSFlow is coronary sinus flow in mL/min/g left ventricle. PCr/ATP is determined by magnetic resonance spectroscopy.

* $p < 0.01$ *vs* baseline.

† $p < 0.05$ *vs* baseline.

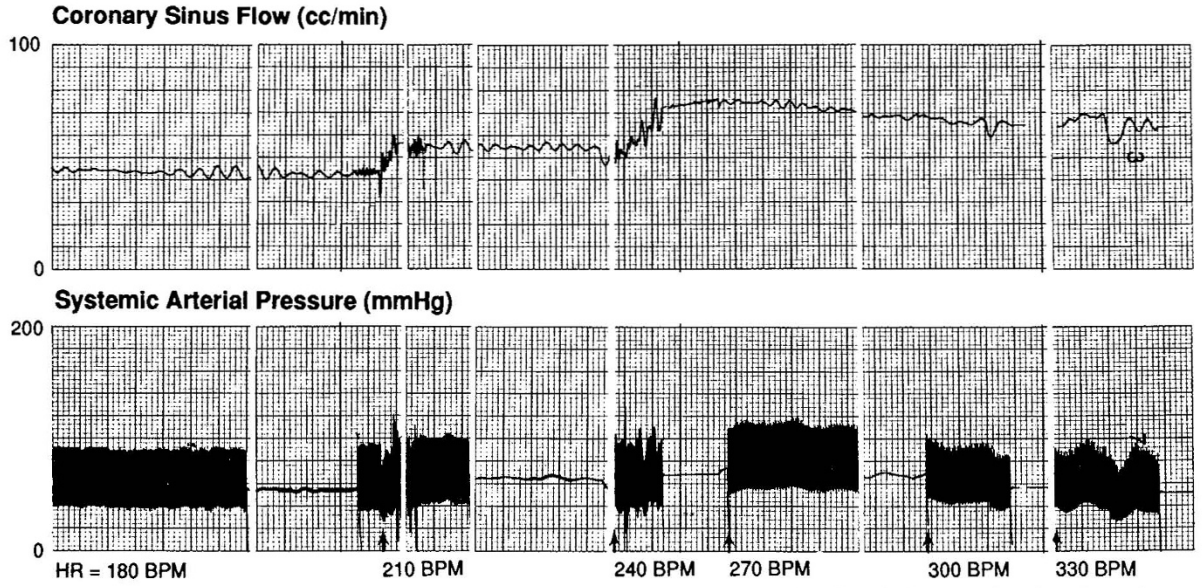

Figure 1. The tracings represent simultaneous recording of systemic arterial pressure and coronary sinus flow during alterations in atrial pacing rate. Systemic systolic/diastolic pressure is intermingled with the mean pressure. Breaks in the tracing occurred during blood sampling. Changes in heart rate are noted at the bottom. Coronary sinus flow and arterial pressure rise with each pacing increase until 300 bpm. At 330 bpm, previously steady coronary flow rate becomes unstable and decreases as does blood pressure.

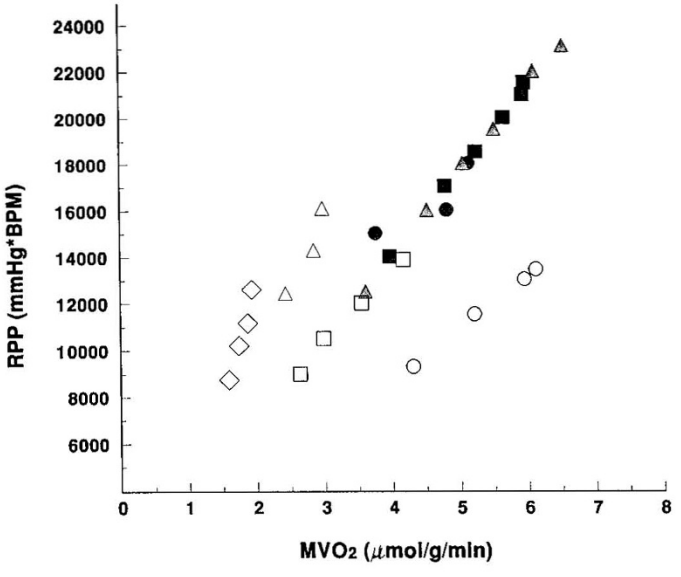

Figure 2. RPP *versus* MVO_2 (μmol/g/min) is plotted for seven sheep during incremental pacing rate increases. Each symbol represents a different animal. Linear regression analysis yields the following equation: RPP = 6367(±1552)MVO_2 + 2047(±347); $r = 0.75$.

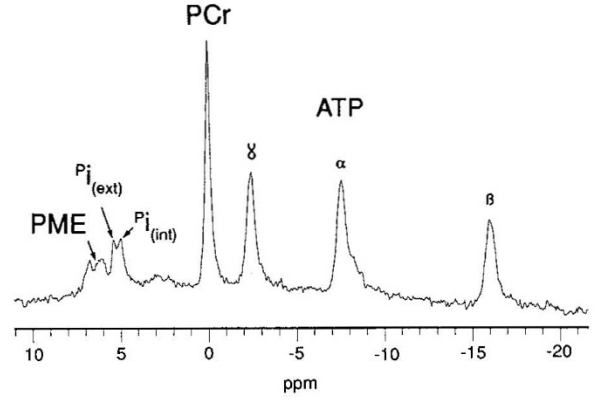

Figure 3. A representative ^{31}P spectrum of a newborn lamb heart is shown. This spectrum is the sum of 120 acquisitions, sweep width 5000 Hz, with 5-Hz line broadening as the only postprocessing applied. Labeled peaks are phosphomonoesters (*PME*), extracellular inorganic phosphate ($P_{i(ext)}$), intracellular inorganic phosphate ($P_{i(int)}$), PCr, and the three peaks of ATP, γ, α, and β.

High-energy phosphates. Figure 3 illustrates a typical ^{31}P spectrum representing the average of 120 acquisitions obtained in 4 min. Paired *t* test analysis of deconvolution- and integration-derived PCr and β-ATP peak areas shows that a significant decrease in PCr/ATP occurs during peak oxygen consumption with a complete recovery obtained upon return to baseline pacing rates. β-ATP area remained stable during the paced tachycardia. Sufficient signal to noise ratio was not obtained in these experiments to determine whether the P_i/ATP was altered significantly. Figure 4 illustrates a baseline spectrum in *A*, whereas *B* represents a spectrum obtained at peak. Both spectra represent the sum of 120 acquisitions with 10-Hz line broadening applied. *C* is the difference spectrum (*B* − *A*). At 0 ppm a decrease in PCr is noted, although at 4.9 ppm a corresponding increase in P_i is apparent. PCr/ATP is plotted against relative MVO_2 in Figure 5 for all seven studies. PCr/ATP is lower at peak oxygen consumption than at baseline in all seven animals using paired *t* test at a significance level of $p < 0.001$. For the purpose of comparison with values from previous work (1, 7), free intracellular ADP concentrations were calculated through the creatine kinase reaction as noted in "Methods." Using the values for intracellular pH (7.10, 7.10, 7.11) determined from the P_i chemical shift and PCr/ATP values of 1.55, 1.36, and 1.67, respectively, from baseline, peak, and recovery periods, ADP values of 37 ± 5, 46 ± 7, and 32 ± 9 μM cell water were obtained. The baseline value differed significantly from peak ($p = 0.03$) but not from recovery.

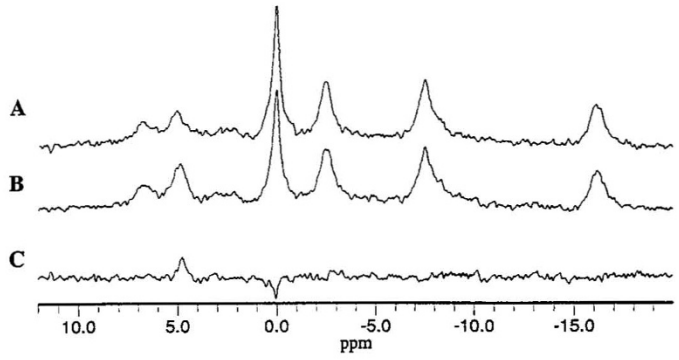

Figure 4. *A* and *B* represent spectra obtained during baseline and peak periods using parameters as described in Figure 3. Ten-Hz line broadening was used. *C* is the differences spectrum ($B - A$). PCr at 0.0 ppm decreases although there is a corresponding increase in P_i at 4.9 ppm.

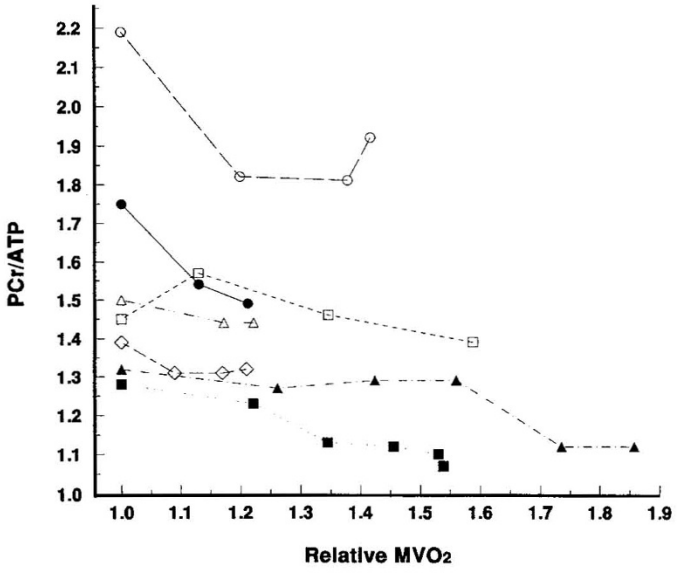

Figure 5. PCr/ATP is plotted *vs* relative MVO_2 ($MVO_2/MVO_{2baseline}$). Oxygen consumption was calculated from coronary sinus flow × coronary arterial-venous oxygen content in seven sheep. Symbols correspond to those in Figure 2.

Substrate uptake. Data from the substrate uptake experiments are presented in Table 2. In this group, the transition from baseline to peak heart rate was accompanied by increased oxygen consumption and uptake of the three measured substrates ($p < 0.05$). However, there were no significant changes in the coronary arterial-venous difference for oxygen or any of these oxidative substrates. Changes in coronary flow ($p < 0.05$) accounted solely for the increased uptake. This also indicates that no change occurred in relative uptake of these substrates and oxygen.

Plasma catecholamines. Fractionated catecholamines were analyzed in three lambs during the pacing protocol to determine whether a change was elicited by increasing the pacing rate. Norepinephrine was the primary circulating catecholamine at 236 ± 28 pg/mL at baseline, with no significant change at peak pacing, 232 ± 27 pg/mL. Epinephrine and dopamine levels were less than 25 pg/mL at both pacing rates. However, epinephrine infusion increased circulating epinephrine levels to 26 000 ± 1 800 pg/mL, which is more than a 100-fold change in total circulating catecholamines. These data indicate that atrial pacing minimally effects circulating catecholamine levels. Furthermore, substantial increases in circulating epipnephrine are required to elevate coronary blood flow and MVO_2 by at least 50% in this open-chest sheep model.

DISCUSSION

This experimental model has been used previously to show that the relationship between high-energy phosphates and MVO_2 changes as a function of development, when cardiac work is altered using a catecholamine stimulus (1, 3). In those studies, levels of the ATP hydrolysis products, ADP and P_i, increase in newborn lambs during moderate or submaximal increases in MVO_2. Even larger increases were not associated with such changes in mature sheep. Surprisingly, this study demonstrates that changes in oxygen consumption during increases in heart rate elicited by short-term atrial pacing are associated with similar decreases in the PCr/ATP ratio and increased calculated ADP levels. Thus, this relationship between high-energy phosphates and oxygen consumption is not restricted to specific conditions using high-dose catecholamine stimulus or large increases in oxygen consumption. Therefore, a true alteration in regulation of myocardial respiration may occur during maturation. The transient nature of these alterations and their association with increased MVO_2 is demonstrated by the return to baseline levels in the recovery period. Previous study in this animal model has also shown that newborn lamb myocardial phosphate levels do remain stable during long pacing periods at relatively constant rates (7), similar to those used at baseline in these experiments. These data reinforce the conclusion that the increases in heart rate with accompanying increases in oxygen consumption are responsible for the associated change in myocardial phosphates, and that this alteration is not a time-dependent effect of pacing alone. Conceivably, relative myocardial underperfusion during paced tachycardia could occur and be responsible for these PCr/ATP decreases. Heineman and Balaban (11) have clearly demonstrated that this phenomenon is associated with decreased perfusion pressure, RPP, coronary sinus flow, and

Table 2. *Myocardial substrate uptake during baseline and peak oxygen consumption* (n = 4)

Heart rate (bpm)	Coronary sinus flow (mL/min/g)	Coronary A-V content (mM)			
		O_2	Lactate	Glucose	FFA
150	0.70 ± 0.04	6.5 ± 0.2	0.70 ± 0.02	0.46 ± 0.08	0.11 ± .003
240	0.91 ± 0.08*	6.4 ± 0.2	0.71 ± 0.03	0.51 ± 0.09	0.11 ± .004

Data are expressed as mean ± SEM. A-V, arterial-venous.
* $p < 0.05$.

PCr/ATP ratio. For these reasons, only animals that demonstrated blood pressure stability were included in this study. Furthermore, Fujii *et al.* (12) have recently shown that no change in endocardial flow occurs with atrial pacing in unanesthetized lambs, although epicardial flow increases significantly. This finding is difficult to extrapolate to our study, which used younger anesthetized lambs. However, it does support the belief that endocardial ischemia did not occur during the experiments included in our analysis.

For the purposes of this study, a hypothesis was developed that predicted that alterations in myocardial phosphates during epinephrine infusions were due to exogenous catecholamine influence on substrate supply and utilization and not strictly due to oxygen consumption changes. Formulation of this hypothesis was based on studies that evaluated the influence of substrate alterations on phosphorylation potential (5, 6). From *et al.* (5), using perfused rat hearts, demonstrated that at constant work state alteration of mitochondrial NADH through carbon substrate manipulation results in changes in phosphorylation potential without commensurate change in MVO_2. Supportive data in intact animals were published by Kim *et al.* (6), who demonstrated an increase in phosphorylation potential during fatty acid inhibition by ketone bodies, which was not accompanied by changes in oxygen consumption. Major alterations in circulating substrate levels are well documented to occur during exogenous epinephrine infusion (6, 13), even at doses too low to elicit hemodynamic changes (14). Catecholamines increase glycolysis and lipolysis in various tissues with resulting increases in circulating plasma levels of FFA glucose and lactate (15, 16). The effects of epinephrine infusion on these circulating metabolites in sheep have also been shown to be age dependent (14, 17). For example, circulating lactate levels are 5- to 6-fold higher in newborns than in adults, and uptake rises dramatically with epinephrine infusion (14). Furthermore, developmental adaptations have been noted in the heart's response to manipulation of substrate supply. Lactate in particular markedly reduces palmitate oxidation in both the newborn and fetal heart (18, 19), a response that theoretically should reduce both NADH/NAD and phosphorylation potential (5).

In contrast, recent investigation in the isolated working rabbit heart has shown that increasing heart rate and oxygen consumption by atrial pacing does not alter the NAD(P)H redox state (20). Furthermore, data provided in this study indicate that no reapportionment of uptake from three major substrates occurs with the modest increases in oxygen consumption induced by paced tachycardia. Substrate uptake is not synonymous with substrate oxidation. However, in the well-oxygenated heart, reapportionment of substrate uptake usually affects substrate utilization within the heart (5, 21). Lactate uptake in particular can be related to the oxidation rate, because release of pyruvate or alanine does not occur *in vivo* and lactate is not converted to glucose in the heart, thereby directing all lactate into oxidation (6, 21). Therefore, lack of substrate reapportionment strongly supports the contention that no substantial change in the source of mitochondrial reducing equivalents, and thus NADH/NAD, occurs during paced tachycardia.

Therefore, our results do not support the formulated hypothesis. Thus, it is assumed that the decrease in PCr/ATP and increase in calculated ADP can be related directly to changes in oxygen consumption, and that the ATP hydrolysis products more directly participate in respiratory regulation in the newborn *in vivo*. A Michaelis-Menten model of myocardial respiratory control can be formulated using a simple random bireactant system and the following equation as described by Katz *et al.* (22):

$$MVO_2 = V_{max} \frac{[ADP][P_i]/K_{ADP}K_{Pi}}{1 + [ADP]/K_{ADP} + [P_i]K_{ADP} + [ADP][P_i]/K_{ADP}K_{Pi}}$$

where V_{max} is the maximum respiratory rate, K_{ADP} is the Km of ADP, and K_{Pi} is the Km of P_i. The published Km values in isolated mitochondria are ~20 μM and ~800 μM for ADP (4) and P_i (23), respectively. However, for this model in newborn sheep myocardium *in vivo*, it is assumed that the Km are near the reported resting values, 3.7 mM for P_i (7) and 30 μM for ADP (1, 3). V_{max} is assumed to be oxygen 20 μmol/g/min, a value extrapolated from oxygen consumption studies performed in exercising lambs (24). [ADP] is calculated from the creatine kinase equilibrium equation. Also necessary for construction of this model are assumptions that ATP remains constant and $\Delta PCr = \Delta P_i$, which are assertions consistent with many NMR studies (1, 25). The relationship between ADP and MVO_2 for this model is plotted in Figure 6, but a corresponding curve can be computed for P_i *versus* MVO_2. Also shown are baseline and peak data from the pacing experiments and previously published epinephrine studies performed in both newborn and mature lambs *in vivo*. The $\Delta ADP/\Delta MVO_2$ for the Michaelis-Menten model corresponds to the actual data from both series of newborn lamb experiments. This comparison

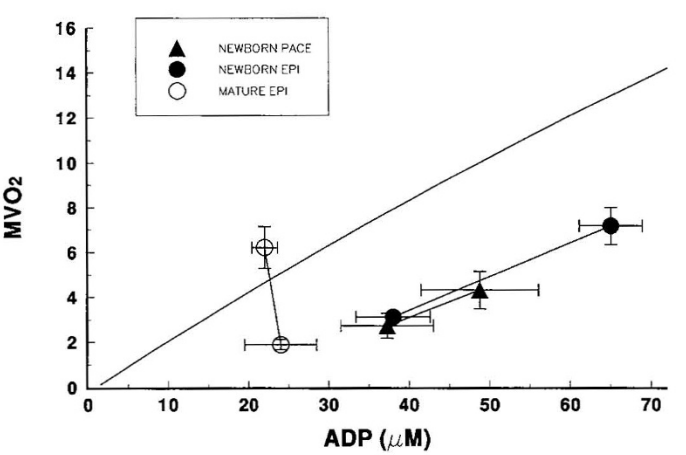

Figure 6. MVO_2 (μmol/g/min) *vs* ADP is plotted for a simple bireactant kinetic model (*solid curve*) assuming Km for ADP and P_i are near the resting concentrations in newborn lamb myocardium. Also shown are summations of baseline and peak data (connected by lines) derived from pacing-induced increases in MVO_2 in newborn lambs, and previously published baseline and peak data for epinephrine-stimulated increases in both newborn (*NEWBORN EPI*) and mature sheep (*MATURE EPI*) (3). A corresponding curve can be generated for MVO_2 *vs* P_i. The directional change in ADP during oxygen consumption increase, demonstrated by the newborn data, is consistent with the Michaelis-Menten model. The data from the mature sheep hearts are clearly different and are not consistent with this model.

indicates that a simple kinetic mode of respiratory control is plausible in the newborn lamb heart, although several studies demonstrate that such a mechanism is probably not operative in the more mature animal (1, 3, 22) during aerobic metabolism. Figure 6 emphasizes this point, inasmuch as the relationship between ADP and MVO_2 in mature sheep hearts during epinephrine stimulation clearly differs from those delineated for both the predicted model and newborn sheep.

Obviously, it would be purely speculative to suggest why this relationship differs in the newborn. Recent theories of myocardial respiratory control dismiss previously simplistic models (26). Instead, complex interactions of biochemical pathways including those that influence rates of mitochondrial substrate supply and oxidation, such as mitochondrial calcium transport, have been implicated as multifaceted regulatory mechanisms. Many, and perhaps most, of these mechanisms undergo developmental changes, which may influence the relationship of the ATP hydrolysis products with oxygen consumption (27). Further analyses of these reactions are currently under study.

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