Myocardial Carbohydrate, Ketone, and Fatty Acid Uptake in Conscious Lambs with Aortopulmonary Shunts¹

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ABSTRACT. A left to right shunt increases myocardial work and is often accompanied by increased catecholamine levels. Because both increased myocardial work and increased catecholamine levels may induce increased fatty acid utilization, which could increase resting myocardial oxygen consumption and therefore unfavorably affect coronary reserve, we studied myocardial uptake of glucose, pyruvate, lactate, β -OH-butyrate, acetoacetate, FFA, and triglycerides in 12 7-wk-old lambs with aortopulmonary left to right shunts ($58 \pm 2\%$ of left ventricular output, mean $\pm$ SEM) and in 10 control lambs 2 wk after surgery. Despite the shunt, systemic blood flow in the shunt lambs was maintained at the same level as in the control lambs. This was accomplished by an increased heart rate and stroke volume. Furthermore, the shunt was accompanied by an increased myocardial oxygen consumption in the shunt lambs (834 ± 70 versus $528 \pm 43 \mu\text{mol O}_2 \cdot \text{min}^{-1} \cdot 100 \text{ g}^{-1}$; $p < 0.05$). There were no significant differences in arterial substrate concentrations between the two groups. The same was true for arteriovenous differences across the myocardium, with the exception of lactate, which was substantially higher in shunt than in control lambs (72 ± 25 versus $18 \pm 23 \mu\text{mol/L}$; $p < 0.05$). As a consequence, myocardial lactate uptake in the shunt lambs was increased 15-fold (18 ± 6 versus $1 \pm 2 \mu\text{mol} \cdot \text{min}^{-1} \cdot 100 \text{ g}^{-1}$; $p < 0.02$), whereas uptake of the other substrates merely paralleled the increased myocardial blood flow. Our data demonstrate that myocardial substrate uptake is not substantially different between shunt and control lambs, with the exception of lactate, of which the extraction is 10-fold higher than in control lambs. We speculate that the increased myocardial lactate utilization may reflect an increase of lactate and pyruvate dehydrogenase activities. (*Pediatr Res* 32: 27–32, 1992)

Abbreviations

LDH, lactate dehydrogenase
PDH, pyruvate dehydrogenase
OER, oxygen extraction ratio

A left to right shunt through a ventricular septal defect or ductus arteriosus imposes an increased work load on the heart because of its effect on left ventricular wall stress, external work, contractility, and heart rate (1–3). This effect is accompanied by an increased myocardial oxygen consumption (3), which reflects increased oxidation of substrates to meet the increased ATP demand (4). Often, an increased work load leads not only to increased substrate uptake, but also to a change in the pattern of myocardial substrate utilization (5), as has been shown in hearts with increased afterload (6), contractility (7), and thyroxine-induced volume load (8, 9) and during exercise (5, 10–12). Furthermore, catecholamines are often increased in the presence of a substantial left to right shunt (13), and they are also known to affect myocardial metabolism (14, 15). Through their effect on myocardial performance, catecholamines stimulate glucose and fatty acid utilization by the myocardium, and, through activation of lipoprotein lipase, they enhance fatty acid utilization (5, 14–16).

These considerations suggest that a left to right shunt could lead to a change in myocardial substrate metabolism. A shift toward increased fatty acid uptake could increase myocardial oxygen consumption because of the low phosphorylation/oxidation ratio, *i.e.* mol of ADP phosphorylated per mol O consumed (4, 17, 18). This would create an unfavorable situation because myocardial oxygen consumption is already high because of the increased work load of the heart. An added increase in oxygen demand would have to be met by an increased myocardial blood flow and would thus result in a decreased coronary blood flow reserve. In addition to the effects on myocardial oxygen consumption mentioned above, adverse effects of enhanced fatty acid uptake on myocardial performance have also been reported in isolated hearts (19–22). If, instead of an increase in fatty acid uptake, a shift toward increased glucose or lactate uptake would occur, as occurs during the increased myocardial work accompanying exercise (10–12, 17), this would probably not be unfavorable. This is supported by the finding that enhanced glucose utilization decreases myocardial oxygen consumption (23). Insight into the metabolism of hearts with an aortopulmonary left to right shunt could add to the understanding of the pathophysiology of subjects with such a shunt.

Accordingly, the objective of this study was to analyze myocardial substrate uptake in lambs with an aortopulmonary left to right shunt by measuring the myocardial uptake of glucose, pyruvate, lactate, β -OH-butyrate, acetoacetate, FFA, and triglycerides.

MATERIALS AND METHODS

We studied 22 7-wk-old lambs of mixed breed with documented dates of birth. They were divided into two groups: 12 lambs with an aortopulmonary left to right shunt and 10 lambs

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without a shunt, which served as controls. Until the day of study, each lamb remained with its mother.

Surgical procedure. Surgical preparation, catheter care, and antibiotic administration were performed as described previously (24). In brief, after induction of halothane anesthesia, we performed a thoracotomy through the 4th intercostal space and sutured a Goretex conduit (inner diameter 6 mm; W. L. Gore and Associates, Inc., Flagstaff, AZ) between the descending aorta and the main pulmonary artery at the level of the fibrotic string of the ductus arteriosus. Precalibrated electromagnetic flow transducers (inner diameter 10–15 mm; Skalar Medical, Delft, The Netherlands) were placed around the ascending aorta just above the coronary arteries and around the pulmonary artery proximal to the conduit. Polyvinyl catheters were placed in the ascending aorta, pulmonary artery, right ventricle, left and right atrium, and coronary sinus. In the coronary sinus, which was cannulated via the left azygos vein, blood predominantly from the left ventricular free wall drains (25). Finally, the chest wall was closed in layers, and the catheters and flow probe cables were led through a subdermal tunnel to a cloth pouch sewn to the left flank of the lamb. For the control lambs, surgical instrumentation was the same except for the conduit, the flow transducer around the pulmonary artery, and the right ventricular catheter.

Experimental protocol. On the day of study, 12 ± 1 d after surgery, the lamb was weighed at 0730 h and brought to the experimental room, where it was allowed to stand. To prevent interference with FFA metabolism, the heparin fluid was carefully aspirated from the catheters. Measurements were started after a 2–3 h habituation period, but only when the lamb stood quietly. Throughout the experiment and the 2–3 preceding h, the lamb was allowed no food (25). Systemic and pulmonary blood flows, as well as aortic, pulmonary arterial, and left and right atrial pressures, were measured every 5 min over a 30-min period. At 15 and 30 min, blood samples (0.7 mL) were withdrawn from the aortic and coronary sinus catheters with a dry, heparinized syringe. O_2 saturation was determined in all samples; Hb concentration, pH, PCO_2 , PO_2 , and plasma HCO_3^- concentration were determined only in the sample from the aorta. Aortic hematocrit was determined by the microcapillary method in duplicate. At 30 min, blood samples (9 mL) were drawn from the aortic and coronary sinus catheters to determine the myocardial substrate concentrations in triplicate. One additional blood sample (4 mL) was drawn from the aortic catheter to determine catecholamine concentrations. Immediately after collecting these blood samples, we injected radioactive microspheres labeled with either ^{141}Ce , ^{51}Cr , ^{103}Ru , or ^{95}Nb (NEN-Trac; Dupont Co., Biotechnology Systems, Wilmington, DE) into the left atrium while a reference sample was simultaneously drawn with a pump (Harvard Apparatus Co., Millis, MA) from the aortic catheter into a preweighed, heparinized syringe for 1.25 min at a rate of 6 mL/min (26).

Two identical experiments were performed in each lamb with a 2-d interval. In two shunt lambs, no second experiment could be performed because of obliteration of the coronary sinus catheter.

Measurements and calculations. The precalibrated electromagnetic flow transducers were connected to Skalar MDL 400 flow meters. Systemic blood flow of the shunt lambs was obtained from the pulmonary arterial flow transducer, which is situated proximal to the anastomosis of the shunt and the pulmonary artery and thus measures systemic venous return (see Fig. 1 from Ref. 3). Pulmonary blood flow of the shunt lambs was obtained from the aortic flow transducer, which is situated proximal to the anastomosis of the shunt and the aorta and thus measures pulmonary venous return. Systemic blood flow of the control lambs was obtained from the aortic flow transducer. The position of the aortic flow transducer in both groups was distal to the origin of the coronary arteries. To obtain total left ventricular output, the flow signal was later modified by adding coronary blood flow obtained with the microspheres (27). Heart rate was

obtained from the blood flow signal. Aortic and left atrial pressures were measured with Gould P23 ID pressure transducers (Spectramed Inc., Oxnard, CA) that were referenced to atmospheric pressure with zero obtained with the pressure transducer at right atrial level. All variables were recorded on an Elema Mingograf 800 ink-jet recorder (Siemens-Elema AB, Solna, Sweden). O_2 saturation was determined in duplicate with an OSM2 hemoximeter (Radiometer, Copenhagen, Denmark). pH, PCO_2 , PO_2 , and plasma HCO_3^- concentrations were determined with an ABL-2 blood gas analyzer (Radiometer). Hb concentration was determined with the methemoglobinocyanide method (28). Left to right shunt flow was obtained by subtracting systemic from pulmonary blood flow. Left to right shunt fraction was calculated by dividing left to right shunt flow by pulmonary blood flow. Blood O_2 concentration was calculated as the product of O_2 saturation, Hb concentration, and an Hb binding capacity of 1.36 mL O_2 /g Hb (29).

Blood flow to the myocardium was determined with two of the four radionuclide-labeled 15- μ m microspheres injected in random order. After the second experiment, the lamb was killed with an overdose of i.v. pentobarbital. Then the heart, cerebral hemispheres, and kidneys were removed and weighed. The heart was cleared of its pericardium, great vessels, chordae, and epicardial fat. The left ventricular free wall was separated from the rest of the heart and both were weighed with an accuracy of 1 mg. Radioactivity in the left ventricular free wall, the rest of the myocardium, hemispheres, kidneys, and reference samples was determined in a Beckman 9000 gamma counter (Beckman Instruments, Fullerton, CA). Organ blood flows were calculated from the radioactivity counts in the tissue samples and arterial reference samples with the aid of a special computer program (28). Blood flows were expressed in $mL \cdot min^{-1} \cdot 100 g^{-1}$ wet weight. Adequate mixing of the microspheres in each lamb was achieved by ascertaining that blood flow per 100 g of tissue to the two cerebral hemispheres, as well as to the two kidneys, did not differ by more than 10% (26).

Concentrations of glucose, pyruvate, lactate, β -OH-butyrate, and acetoacetate were determined in triplicate in whole blood by enzymatic methods (30). Immediately after sample collection, the blood (4.5 mL) was transferred to a tube containing a dash of NaF and carefully mixed to prevent glycolysis. Then the blood was deproteinized with perchloric acid. After subsequent neutralization to pH 7 with KOH and morpholinopropansulfonic acid solution, the mixture was centrifuged and the supernatant removed to determine the substrate concentrations (30).

Concentrations of FFA, total glycerol, and free glycerol were determined in triplicate in plasma. Immediately after sample collection, the blood (4.5 mL) was transferred to a chilled tube and centrifuged. The plasma was removed and stored without delay at $-70^\circ C$, pending determination of the substrate concentrations. FFA concentration was determined enzymatically with a commercial kit (NEFAC; Wako Chemicals GmbH, Neuss, Germany) (31). Total glycerol concentration was determined similarly (Test-Combination Triglyceride; Boehringer Mannheim GmbH, Mannheim, Germany). Free glycerol was also determined enzymatically (30). Plasma concentrations were converted to blood concentrations through multiplication with the following factor: $[100 - \text{hematocrit} (\%)]/100$. The triglyceride kit measures total glycerol concentration after hydrolysis of triglycerides. To obtain the triglyceride concentration, we subtracted free glycerol from total glycerol concentration.

Because coronary sinus blood consists predominantly of venous blood from the left ventricular free wall, we calculated myocardial O_2 consumption of the left ventricular free wall as the product of aortocoronary sinus O_2 concentration difference and blood flow to the left ventricular free wall (25) obtained with the radioactive microspheres. Similarly, we calculated myocardial substrate uptake as the product of aortocoronary sinus substrate concentration difference and blood flow to the left ventricular free wall. Substrate extraction ratio was calculated by

dividing aortocoronary sinus difference by arterial substrate concentration.

To assess the relative importance of each substrate for myocardial oxidative metabolism, we calculated the OER (10, 17). It was calculated by assuming that during a steady state all of the substrate taken up by the myocardium is combusted, using the formula $OER = (ACS_{\text{substrate}} \times N) / (ACS_{\text{oxygen}})$, in which ACS is the aortocoronary sinus concentration difference and N represents the number of mol of oxygen required to completely combust 1 mol of substrate (25). $N_{\text{glucose}} = 6$, $N_{\text{pyruvate}} = 2.5$, $N_{\text{lactate}} = 3$, $N_{\beta\text{-OH-butyrate}} = 4.5$, $N_{\text{acetoacetate}} = 4$, $N_{\text{FFA}} = 25$, and $N_{\text{triglycerides}} = 75$ (10, 17). N is obtained from the stoichiometric relationship between oxygen and the respective substrate when expressed in equivalent molar concentrations.

Plasma noradrenaline and adrenaline concentrations were determined by HPLC with electrochemical detection (32). After sample collection, the blood was centrifuged in a cooled (4°C) centrifuge. The thrombocyte-poor plasma was fortified with the antioxidant glutathione and stored at -20°C pending determination.

Statistical analysis. Data are expressed as means $\pm$ SEM. The differences between shunt and control lambs were evaluated with the Mann-Whitney test (33). Correlation coefficients and regression equations were calculated and are reported according to standard techniques (33, 34). $p \leq 0.05$ was considered significant.

RESULTS

There were no substantial differences in weight (12.7 ± 0.6 versus 12.7 ± 0.8 kg) or age (47 ± 1 versus 50 ± 1 d, $p < 0.05$) between shunt and control lambs. The left to right shunt led to significant hemodynamic differences between shunt and control lambs at rest (Table 1), differences that were similar to those reported previously from our laboratory (3, 24). Aortic systolic pressure in the present study was not significantly lower than in control lambs ($p = 0.07$), unlike mean aortic pressure (65 ± 1 versus 71 ± 2 mmHg, $p < 0.01$). Left ventricular mass of the

shunt lambs was higher than that of the control lambs (29 ± 2 versus 24 ± 2 g, $p < 0.05$). Plasma noradrenaline and adrenaline concentrations were higher in shunt than in control lambs, but these differences did not reach statistical significance ($p = 0.08$ and 0.07 , respectively). The power for the latter two tests, however, was low: 0.36 and 0.28, respectively (33).

Arterial oxygen concentration was lower in shunt than in control lambs (Fig. 1) due to the lower Hb concentration and oxygen saturation (Table 1). Myocardial oxygen extraction ratio did not differ between the two groups, so the arteriovenous oxygen concentration difference across the myocardium was lower than in control lambs. However, left ventricular oxygen uptake in the shunt lambs was significantly higher than in control lambs as a consequence of the considerably higher myocardial blood flow. The increased myocardial oxygen consumption must be mainly due to the increase in heart rate, inasmuch as there was no difference in oxygen uptake per beat (5.2 ± 0.5 versus 5.1 ± 0.6 $\mu\text{mol O}_2 \cdot \text{beat}^{-1} \cdot 100 \text{ g}^{-1}$). The effects of increased left ventricular stroke volume and end-diastolic pressure on external work and wall stress are counteracted by myocardial hypertrophy and decreased aortic pressure, resulting in a normalized myocardial O_2 consumption per 100 g per beat (28).

Arterial substrate concentrations were not significantly different between the two groups (Fig. 1). Arteriovenous lactate concentration difference was higher in shunt than in control lambs. As a consequence, myocardial lactate extraction ratio was also higher in shunt than in control lambs ($8 \pm 3\%$ versus $1 \pm 2\%$; $p < 0.05$). As for the other substrates, there were no significant differences between the two groups in arteriovenous concentration differences across the myocardium. Myocardial uptake of lactate was substantially higher in shunt than in control lambs due to the increased arteriovenous difference and the 2-fold increase in myocardial blood flow. Unlike oxygen uptake, myocardial lactate uptake increased more than heart rate so that lactate uptake per beat was still higher in shunt than in control lambs (107 ± 33 versus 16 ± 25 $\text{nmol} \cdot \text{beat}^{-1} \cdot 100 \text{ g}^{-1}$, $p < 0.05$). Inasmuch as the arteriovenous difference across the myocardium for the other substrates was similar in the two groups, their myocardial uptake paralleled the increase in myocardial blood flow in the shunt lambs, as was the case for myocardial oxygen consumption. The increased uptake of several substrates did not, however, reach statistical significance, a result for which we hold the spread of the values responsible. The variable nature of myocardial metabolism ["the heart is a scavenger" (17)] contributes to this spread because it varies according to arterial substrate concentrations and the between-substrate concentration relationships (4, 5).

Because myocardial lactate uptake in the shunt lambs was substantially more increased than oxygen uptake, the OER for lactate was considerably higher in shunt than in control lambs (Fig. 2). OER for total fatty acids (sum of FFA and those fatty acids derived from triglycerides) amounted to approximately 0.5 and that for ketone bodies amounted to 0.2, in shunt as well as in control lambs. OER for glucose was 0.10 in shunt and 0.05 in control lambs. The sum of the OER of the different substrates did not add up to 1. For both groups it was not significantly different from 1, yet it could clearly be distinguished from 0 (shunt, $p < 0.001$; control, $p < 0.01$).

There was a significant correlation between arterial $\beta\text{-OH-butyrate}$ concentrations and arteriovenous differences ($r = 0.67$, $p < 0.0001$). An even higher correlation was found between arterial concentrations and OER of $\beta\text{-OH-butyrate}$ ($r = 0.82$; $p < 0.0001$) (Fig. 3). Subdivision into shunt and control lambs showed significant correlations in both groups.

DISCUSSION

The overall pattern of myocardial substrate uptake in shunt lambs is not substantially different from that in control lambs. Myocardial uptake of the substrates in the shunt lambs parallels

Table 1. Data for shunt and control lambs*

	Shunt	Control
Blood flow ($\text{mL} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$)		
Systemic	121 ± 4	120 ± 4
Pulmonary	297 ± 12	120 ± 4 †
Heart rate (beats/min)	164 ± 5	108 ± 5 †
Pressure (mm Hg)		
Systolic aortic	83 ± 2	88 ± 2
Left atrial	15 ± 1	4 ± 1 †
L. ventricular stroke volume (mL/kg)	1.83 ± 0.06	1.15 ± 0.06 †
Hb (g/L)	93 ± 1	101 ± 3 †
O_2 saturation (%)		
Aortic	89 ± 1	92 ± 0 †
Coronary sinus	28 ± 3	24 ± 3
Myocardial blood flow in left ventricular free wall ($\text{mL} \cdot \text{min}^{-1} \cdot 100 \text{ g}^{-1}$)	243 ± 15	129 ± 9 †
Arterial pH	7.41 ± 0.01	7.44 ± 0.01
Arterial PCO_2 (mm Hg)‡	41 ± 1	38 ± 1 †
Arterial PO_2 (mm Hg)‡	102 ± 2	108 ± 2
Arterial HCO_3^- (mmol/L)	24.7 ± 0.6	24.3 ± 0.6
Arterial noradrenaline (nmol/L)§	16 ± 4	8 ± 2
Arterial adrenaline (nmol/L)§	2.1 ± 0.5	1.2 ± 0.4

* Values are means $\pm$ SEM; $n = 22$ measurements in 12 shunt lambs and $n = 20$ measurements in 10 control lambs. L. ventricular, left ventricular.

† $p < 0.05$, shunt vs control.

‡ Conversion factor mm Hg to kPa: $\times 0.1333$.

§ Conversion factor nmol/L to ng/L: $\times 168$ and $\times 182$, for noradrenaline and adrenaline, respectively.

Fig. 1. Arterial O₂ and substrate concentrations and arteriovenous difference across and uptake by the left ventricular free wall of shunt (hatched bars, $n = 22$ measurements in 12 lambs) and control lambs (open bars, $n = 20$ measurements in 10 lambs) (means $\pm$ SEM). Asterisks indicate a significant difference between shunt and control lambs.

the increased myocardial blood flow. The only exception, however, is lactate, the uptake of which is 15 times higher than in control lambs. This is the result not only of an increased myocardial blood flow but also of an additionally increased lactate

Fig. 2. OER of the various myocardial substrates for shunt and control lambs. Asterisk indicates a significant difference between shunt and control lambs.

Fig. 3. Arterial β -OH-butyrate concentration versus β -OH-butyrate OER in shunt (open circles) and control lambs (closed circles). There was a significant correlation [$r = 0.82$, $p < 0.0001$, $n = 42$, $y = 0.28 \cdot 10^{-3} (\pm 0.32 \cdot 10^{-4}, \text{SEM}) \cdot x + 0.050 (\pm 0.017)$, residual variance 0.0018]. There was also a significant correlation between arterial β -OH-butyrate concentration and arteriovenous β -OH-butyrate difference [$r = 0.67$, $p < 0.0001$, $n = 42$, $y = 0.24 (\pm 0.04) \cdot x + 41 (\pm 22) \mu\text{mol/L}$, residual variance 3154].

extraction by the myocardium.

An increased myocardial lactate utilization has been previously demonstrated at rest (35, 36), during exercise (4, 10–12, 17, 35, 37–39), or during septic shock (40). However, a common feature in all of these studies is an increased arterial lactate concentration. This enhances myocardial lactate uptake because arterial substrate concentration is a major determinant of myocardial substrate selection (4, 5, 16, 17).

The present data now demonstrate an increased lactate uptake by the myocardium in the shunt lambs, whereas arterial lactate concentration is equal to that in control lambs. This indicates that there is an intrinsic difference between the myocardium of shunt and control lambs. It is likely that the increased arteriovenous difference for lactate across the myocardium of the shunt lambs is due to an increase in enzyme activity leading to increased myocardial lactate extraction and/or more efficient use of intracellular lactate (11, 41). Probably, PDH or LDH-H activity, or both, are increased, inasmuch as these enzymes control the pathway between lactate uptake and acetyl-CoA formation (5, 16).

PDH activity increases by catecholamine stimulation (42, 43) and increased myocardial work load. LDH can adapt itself to changing circumstances by changing its isoenzyme profile (44–

46). The H-subunit activity distribution in the heart parallels the amount of work performed by the different regions of the heart. Its activity is for instance higher in left than in right ventricular myocardium (47). It is therefore conceivable that the myocardium in shunt lambs adapts itself to the chronically increased work load by changing its LDH isoenzyme profile toward increased H-subunit activity. In combination with increased PDH activity, this could lead to increased lactate utilization by the myocardium. This is in agreement with studies in dogs and humans that have shown that the enhancing effect of acute (37) as well as chronically (39) increased myocardial work load on lactate uptake is in addition to the effect of increased arterial lactate concentration. It also fits the finding of a decreased K_m for the conversion of lactate into pyruvate in rat heart homogenates after 10 wk of treadmill training, suggesting that a heart with a chronically increased work load is more capable of utilizing lactate as a fuel for energy metabolism (39, 48).

The contribution of lactate to myocardial oxidative metabolism is small, and in absolute values the difference between shunt and control lambs is small. However, despite the large spread in the values, which is inherent in myocardial metabolism, the difference between the two groups emerges as a significant one. On the clinical significance of increased lactate, we can only speculate. It could be a mere accompanying feature of increased work load, as discussed in the paragraph above. Or it could reflect adaptive changes in PDH or LDH isoenzymes that accompany various states of myocardial (de)compensation (45, 46). Furthermore, myocardial hypertrophy has been demonstrated to be accompanied by abnormal gene expression, leading to synthesis of fetal myocardial protein isoforms (44). The fetal lamb myocardium predominantly uses lactate as a fuel (25). Our findings of increased myocardial lactate uptake in the hypertrophied heart could reflect the presence of fetal myocardial proteins in the shunt lambs.

Increased work load and catecholamines may enhance fatty acid metabolism (5, 15). In the shunt lambs, catecholamine concentration did not quite reach statistical significance ($0.05 < p < 0.10$). The power of the test, however, was low, so that the chance of statistically missing a difference was large. The lack of enhancement of fatty acid uptake may be due to the period of time that the myocardium was exposed to the increased work load. Studies on the effects of increased work load and catecholamines on myocardial metabolism have often dealt with acute effects. Presumably, the myocardium of the shunt lambs adapted during the 2 wk that the shunt was present. Normalization of myocardial metabolism with time during a constant stress after initial change has been demonstrated before: during a 50- or 120-min treadmill run, myocardial lactate and fatty acid uptake acutely changed but tended to return to resting values during the run (11, 12).

It has been reported that in newborn lambs up to 2 wk of age, as well as in adult sheep, glucose and lactate constitute only a minor fraction of total myocardial energy producing substrates (49). Our data confirm this for 7-wk-old lambs. Data with regard to the other substrates that supply myocardial energy in newborns have been lacking up to now (50). Of special interest is the contribution of fatty acids because they do not contribute to myocardial metabolism in the fetal lamb (25), in contrast to the situation in the adult (5). Our data reveal that by 7 wk after birth fatty acids constitute the major substrate for myocardial energy metabolism, as is the case for the adult human heart (5, 16, 17, 19, 50). In addition, ketone bodies form an important source for energy metabolism, accounting for approximately 20% of oxidative metabolism in shunt as well as in control lambs. This is probably due to a higher ketone body concentration in the blood of lambs than in that of human children (~ 0.2 mmol/L), (4, 17, 51) resulting from the difference in gastrointestinal digestion between the human and the ovine species. The fermentation of hay in the stomachs and intestines of sheep produces short-chain fatty acids that are converted to ketone bodies in the liver (51,

52). The presence of hay in the stomachs of all lambs was confirmed at autopsy.

The cumulative OER did not add up to 1. The outcome, however, was not statistically different from 1. The cumulative OER does not necessarily have to equal 1.0 because OER yields approximate values; there is a potential source of error because OER is calculated from four measurements: arterial and coronary sinus concentration of O_2 and substrate (17). Also, it is calculated under the assumption that all substrates taken up by the myocardium undergo complete oxidation in the steady state. Recent isotope-labeled substrate studies have shown that this is not exactly true (41, 53, 54). OER overestimates the glucose utilization because $\sim 13\%$ is released as lactate (41), whereas lactate and fatty acid utilizations are underestimated. Pyruvate utilization may be overestimated because pyruvate is converted to alanine in small amounts (5). Glucose and fatty acids extracted by the myocardium also enter slow turnover pools, probably glycogen and triglyceride pools (41, 53). In a steady state, we assume that equal amounts are added to and released from these endogenous stores. Despite the inaccuracy, OER is a practical means to obtain an impression of substrate utilization.

We speculate that part of the "missing" myocardial substrates in both groups are short-chain fatty acids, predominantly acetate, which are released by carbohydrate fermentation in the stomach (51) and which are not measured by the FFA kit (31). Acetate is taken up and oxidized more readily in the tricarboxylic acid cycle without complex metabolic pathways than, for instance, fatty acids (17, 19, 55). Furthermore, the deficit could reflect substrate use from myocardial glycogen or triglyceride stores. We do not think, however, that this is very likely because the lambs are assumed to be in a steady state (10). The role of endogenous myocardial stores of substrates has not been elucidated. They form a buffer for substrates extracted from the blood and normally do not constitute an important energy source, except in emergencies such as the sudden onset of hard work or of ischemia (17, 56). Moreover, the combined myocardial glycogen and triglyceride stores would be depleted within several hours if 20% of substrates would be retrieved from endogenous stores (56).

In conclusion, the left to right shunt is not accompanied by a substantial change in myocardial substrate uptake pattern. However, myocardial lactate uptake is significantly increased. Because the arterial lactate concentration was not higher in shunt than in control lambs, we hypothesize that the increased myocardial lactate extraction is due to an increased PDH or LDH-H activity, or both.

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