0031-3998/93/3404-0471$03.00/0
PEDIATRIC RESEARCH

Vol. 34, No. 4, 1993
Printed in U.S.A.

Left Ventricular Oxygen and Substrate Uptake in Chronically Hypoxemic Lambs[1]

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ABSTRACT. Myocardial oxygen demand may be increased in chronically hypoxemic lambs because of their increased heart rate. Therefore, we determined whether left ventricular (LV) oxygen supply, oxygen uptake and oxygen demand were proportionally increased in 6-wk-old lambs, after 4 wk of hypoxemia ($n = 15$), as compared with control lambs ($n = 14$). In addition, we determined whether LV glucose, pyruvate, lactate, FFA and ketoacids uptake were altered in hypoxemic lambs, because of alterations in arterial glucose, pyruvate and lactate concentrations, that may occur in hypoxemia. Hypoxemia was induced by the combination of an atrial septal defect and pulmonary stenosis. Arterial oxygen saturation was decreased in hypoxemic lambs (67 ± 8 *versus* 91 ± 3%, $p < 0.001$), Hb concentration was increased, so that arterial oxygen concentration was similar in both groups of lambs. Myocardial mass (61 ± 13 *versus* 44 ± 9 g, $p < 0.001$) and total myocardial blood flow (117 ± 36 *versus* 62 ± 27 mL·min^{-1}, $p < 0.001$) were increased, mainly due to right ventricular hypertrophy. LV oxygen demand, estimated by the rate pressure product (2072 ± 465 *versus* 1467 ± 358 kPa·beat·min^{-1}, $p < 0.001$), and oxygen uptake (723 ± 223 *versus* 556 ± 184 μmol·min^{-1}·100 g^{-1}, $p < 0.05$) were proportionally increased in hypoxemic lambs. LV oxygen supply increased linearly with oxygen uptake ($r = 0.96$) in all lambs, by adjustments in LV blood flow, which was increased in hypoxemic lambs (168 ± 41 *versus* 134 ± 45 mL·min^{-1}·100 g^{-1}, $p < 0.05$). The increase in LV oxygen uptake in hypoxemic lambs was proportional to the increase in heart rate (166 ± 33 *versus* 118 ± 25 beats·min^{-1}, $p < 0.001$). Arterial lactate, pyruvate and β-hydroxybutyrate concentrations were slightly increased in hypoxemic lambs, but LV substrate uptake was practically unaltered as compared with control lambs. FFA and β-hydroxybutyrate contributed most to LV substrate uptake, whereas the contribution of glucose, pyruvate and lactate was negligible. The total oxygen extraction ratios (0.45 ± 0.43 *versus* 0.51 ± 0.50) indicate that approximately 50% of the fuels for the LV were identified. We conclude that LV oxygen supply is matched to increased oxygen demand in chronically hypoxemic lambs, by the increase in LV blood flow. LV substrate uptake is unaltered in hypoxemic lambs; glucose, pyruvate and lactate uptake is negligible, despite an increased arterial pyruvate and lactate concentration. FFA and ketoacid uptake are insufficient to fuel LV oxidative metabolism. (*Pediatr Res* 34: 471–477, 1993)

Abbreviations

LVAVD, left ventricular arteriovenous concentration difference
OER, oxygen extraction ratio

Left ventricular oxygen supply is closely matched to left ventricular oxygen uptake under normal conditions, because the oxygen extraction reserve of the heart is limited. Adjustments in left ventricular blood flow are important to maintain oxygen supply during acute alterations in arterial oxygen saturation or Hb concentration (1–7).

In chronic hypoxemia, the increased Hb concentration compensates for the decreased arterial oxygen saturation (8), so that no adjustments in left ventricular blood flow would be needed to maintain oxygen supply. In chronically hypoxemic adults native to high altitude, left ventricular blood flow, oxygen supply, and oxygen uptake were lower than in adults at sea level (9). In contrast, in young experimental animals exposed to some form of chronic hypoxemia, left ventricular blood flow and oxygen supply were either similar or increased compared with control animals (10, 11), but left ventricular oxygen uptake was not determined in these studies. Because no signs of left ventricular dysfunction are found in chronic hypoxemia, one expects that left ventricular oxygen demand is met by oxygen uptake and that left ventricular blood flow and oxygen supply change in proportion to oxygen uptake. In chronically hypoxemic lambs, heart rate was increased but systemic blood flow was not increased (8). Because heart rate is an important determinant of left ventricular oxygen demand, we hypothesized that left ventricular oxygen uptake would be increased in these lambs and that left ventricular oxygen supply would be increased proportionally by adjustments in the blood flow.

Substrate uptake of the left ventricle in lambs mainly consists of glucose, lactate, fatty acids, and ketoacids (12). In children with cyanotic heart disease, the activity of rate-limiting oxidative enzymes for carbohydrates and fatty acids were similar to that in children with noncyanotic heart disease (13), suggesting that substrate preference of the myocardium is unaltered in chronic hypoxemia. However, substrate uptake may be affected by alterations in substrate supply. Glucose concentration may be increased by impaired insulin release in chronic hypoxemia (14). Glucose supply and uptake by the myocardium was increased during infusion of catecholamines (15). Catecholamine concentrations were either increased or normal in chronic hypoxemia (16–19). In hypoxemic adults native to high altitude, myocardial lactate and pyruvate uptake was increased, and this was linearly

Received November 17, 1992; accepted May 3, 1993.
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Supported by grants from the Netherlands Organization for Scientific Research (NWO) MEDIGON 900-516-089 and from the Netherlands Heart Foundation 87.047).
[1] Presented in part at the Annual Meeting of The Society for Pediatric Research, 1988, Washington, DC.

related to the increased arterial substrate concentrations (20). Therefore, we hypothesized that glucose, pyruvate, lactate, FFA, and ketoacids are the main fuels for the left ventricular myocardium, but that in chronically hypoxemic lambs, glucose, pyruvate, and lactate contribute more to the substrate uptake than they do in control lambs.

This study therefore had a dual purpose. First, we wanted to determine whether left ventricular oxygen demand is increased in lambs after 4 wk of hypoxemia and whether left ventricular oxygen uptake and supply are increased proportionally. Second, we wanted to determine whether glucose, pyruvate, lactate, FFA, and ketoacids mainly fuel the left ventricular myocardium and whether the uptake of glucose, pyruvate, and lactate is increased in chronically hypoxemic lambs.

MATERIALS AND METHODS

Fifteen chronically hypoxemic and 14 control lambs of mixed breed were studied in the 6th to 7th wk of life. All hypoxemic lambs underwent surgery before the 10th d of life, whereas control lambs underwent surgery at least 1 wk before the experiment.

Surgical procedures and postoperative care. Anesthesia was induced by 2–3% halothane in oxygen. The lamb was placed on a warming pad (39°C), intubated, and ventilated with a mixture of halothane (0.5–1.5%), oxygen (40–60%), and room air by a Servo Ventilator 900B (Siemens-Elema AB, Solna, Sweden). Analgesia was maintained with piritramide 10–20 mg intramuscularly and lidocaine hydrochloride (5 $g \cdot L^{-1}$) was administered locally before each skin incision.

The left thoracic cavity was opened in the 4th intercostal space. Polyvinyl catheters (outer diameter 1.5 mm, inner diameter 1.0 mm) were inserted into the ascending aorta through the internal thoracic artery and into the superior vena cava through the internal thoracic vein. The hemiazygos vein was ligated 1–3 cm from its entrance into the pericardium, and a catheter was advanced toward the heart with its tip placed at the confluence with the coronary sinus. Subsequently, the pericardial sac was opened and catheters were inserted through purse-string sutures into the pulmonary artery, the outflow tract of the right ventricle, and the left atrium. In the lambs that were to be made hypoxemic, an atrial septostomy was performed by means of a 5 F balloon-tipped Fogarty catheter (American Edwards Laboratories, Santa Ana, CA) that was advanced toward the heart from a pedal vein. After positioning the tip into the left atrium through the foramen ovale, we inflated the balloon with 1.5–2.0 mL sterile saline solution and rapidly withdrew it into the right atrium, thus tearing the atrial septum. This procedure was repeated 2–3 times. In addition, an inflatable silicone rubber constrictor, inner diameter 8–10 mm (Hazen Everett Co., Teaneck, NJ), was fitted around the main pulmonary artery. The lambs that served as controls underwent the same surgical procedure apart from the atrial septostomy, the constrictor around the pulmonary artery, and the insertion of a right ventricular catheter. In all lambs, an 8 F polyvinyl catheter was placed to drain the left thoracic cavity. All catheters were tunneled to the left flank 5–10 cm caudal of the 4th intercostal space. The thorax was closed in layers and all the catheters, except the one for chest drainage, were filled with a heparin solution (1000 U/mL). All catheters were sealed and protected in a Teflon pouch that was attached to the skin. The left thoracic cavity was aspirated daily for 4–6 d, then the thoracic drain was removed. Daily, the lambs were weighed and the catheters were refilled with fresh heparin solution. Weekly, each lamb was given iron dextran complex equivalent to 200 mg iron intramuscularly.

Induction of hypoxemia was started 3–5 d after surgery. The constrictor around the pulmonary artery was inflated with sterile saline solution (9 $g \cdot L^{-1}$), thus inducing an atrial right-to-left shunt through the atrial septal defect. On the first and second day of inflation, the right ventricular systolic pressure was raised to systemic and suprasystemic levels, respectively. Thereafter, the constrictor was inflated to lower the arterial oxygen saturation to 60–70%. Once this level had been reached, the constrictor was only inflated further to maintain the arterial oxygen saturation within this range.

Experimental protocol. The lambs were allowed to feed until they were separated from their mothers, approximately 3–4 h before the measurements. The lamb was brought to the study area, placed in a canvas sling, and supported in the upright position. Immediately thereafter, the heparin solution was withdrawn from the catheters and discarded, and the catheters were flushed with sterile NaCl solution (9 $g \cdot L^{-1}$) and connected to pressure transducers. Body temperature was monitored with a rectal temperature probe.

Measurements were obtained when the lamb was quiet and at rest. Oxygen uptake was continuously recorded for 30 min; the values obtained at 15 and 30 min were used to represent oxygen uptake. Blood pressures were measured every 5 min in the aorta, pulmonary artery, right ventricle, and the right and left atrium. At 15 and 30 min, blood samples were withdrawn from the aorta, the coronary sinus, and the right ventricle or the pulmonary artery to measure oxygen saturation, Hb concentration, hematocrit, blood gases, and pH. Additionally, at 30 min 7-mL blood samples were withdrawn simultaneously from the ascending aorta and the coronary sinus to measure substrate concentrations and 4 mL of blood were withdrawn from the ascending aorta to measure catecholamine concentrations. Immediately thereafter, blood flow to the heart and other organs was determined by injecting microspheres into the left atrium, while simultaneously a reference sample was withdrawn from the ascending aorta. At the end of the study, the lambs were killed with an overdose pentobarbital. At autopsy, the position of the catheters was verified and organs were taken out to be processed for measurement of radioactivity.

Measurements and calculations. Oxygen uptake was measured by an open flow-through system by means of a Diaferometer MG 4 (Kipp & Zonen, Delft, The Netherlands) that was connected to a Micrograph BD 2 recorder (Kipp & Zonen). Blood pressures were measured by Gould P23 ID transducers (Spectramed Inc., Oxnard, CA) and heart rate was calculated from the phasic aortic pressure tracing. Blood gases were measured on an ABL2 (Radiometer A/S, Copenhagen, Denmark) at 37°C and corrected to actual body temperature. Oxygen saturation, Hb concentration, and hematocrit all were measured in duplicate: oxygen saturation by an OSM2 (Radiometer A/S), Hb concentration by the cyanomethemoglobin method, and hematocrit by the microhematocrit method.

The blood samples that were obtained for measurement of substrate concentrations were immediately divided into two portions. Four mL were mixed in a tube containing some dried NaF to prevent glycolysis. Then the sample was deproteinized with perchloric acid, neutralized with KOH and morpholinopropanesulfonic acid solution, centrifuged, and immediately stored in ice. Glucose, pyruvate, lactate, β-hydroxybutyrate, and acetoacetate concentrations were measured in triplicate with NADP/NADPH-linked enzymatic methods (21). Pyruvate and acetoacetate concentrations were measured the same day; the other samples were stored at −20°C until measurement. The other 3 mL of blood were immediately transferred to a chilled tube containing dried NaF and centrifuged, and the plasma was frozen to −70°C. Plasma FFA concentration was determined enzymatically by means of a commercial kit (NEFAC, Wako Chemicals GmbH, Neuss, Germany) (22). FFA concentration in whole blood was calculated by multiplying plasma concentration with [100 − hematocrit (%)]/100.

Blood samples for catecholamine determination were centrifuged at 4°C, for 10 min. Thrombocyte-poor plasma was transferred to tubes containing glutathione as antioxidant, stored at −20°C, and measured within 7 d by HPLC with electrochemical detection (23).

Microspheres of 15 μm diameter labeled with either ^{141}Ce, ^{103}Ru, ^{52}Cr, or ^{95}Nb (NEN-Trac, DuPont Co., Wilmington, DE) were used. Reference samples were withdrawn from the ascending aorta at a rate 6–7 $mL \cdot min^{-1}$, starting just before and ending at least 45 s after the completion of the microsphere injection. Usually, the withdrawal time was 90 s. The exact withdrawal rate was calculated from the difference of the mass of the syringe before and after obtaining the reference sample and the withdrawal time. Radioactivity was determined in the heart, the brain, and other organs. At autopsy, the heart was weighed and stored in formalin (8%) for 5–8 d. Subsequently, it was stripped of the great vessels, epicardial fat, and chordae, and divided into atrial and ventricular myocardium. The latter was divided into the free wall of the left and right ventricle and the septum. The brain was divided into left and right hemisphere and brain stem. Each part was weighed and counted separately. Radioactivity measurements were performed with a Beckman 9000 multichannel gamma scintillation counter. Blood flows were calculated from the ratio of radioactivity counts in tissue and reference samples times withdrawal rate of the reference sample by using a software package (24). Blood flows were expressed per 100 g of tissue. Adequate mixing of microspheres in each lamb was checked by ascertaining that blood flow per 100 g between left and right cerebral hemisphere did not differ by more than 10% (25).

Blood oxygen concentration was calculated as the product of Hb concentration, oxygen saturation, and an oxygen-binding capacity of 1.36 $mL \cdot g^{-1}$ (26). The systemic blood flow was the oxygen uptake divided by the arterio–mixed venous oxygen concentration difference. Systemic oxygen supply was the product of arterial oxygen concentration and systemic blood flow. Resistance in a vascular bed was calculated as the difference between mean aortic and mean right atrial blood pressure divided by blood flow per 100 g to that vascular bed. For each animal, a mean value of all the cardiovascular and hematologic variables was calculated from the data points obtained during the 30 min of measurement.

The rate pressure product (heart rate times systolic arterial blood pressure) and an estimate of stroke work (stroke volume times systolic arterial blood pressure) were calculated as indices of left ventricular oxygen demand. The LVAVD for oxygen and substrates was calculated as the difference between ascending aortic and coronary sinus concentrations obtained at 30 min. Because the blood sampled from the coronary sinus is mainly derived from the left ventricular free wall (27), we calculated uptake of oxygen and substrates as the product of LVAVD and the blood flow to the free wall of the left ventricle. The OER for each substrate was calculated using the following formula:

$$OER = (LVAVD_{subtrate}/LVAVD_{oxygen}) \times \text{substrate factor}$$

The substrate factor is equal to the amount of oxygen required to completely oxidize 1 mol of substrate; for glucose it was 6; pyruvate, 2.5; lactate, 3; acetoacetate, 4; β-hydroxybutyrate, 4.5; and FFA, 25 $mol \cdot mol^{-1}$.

Statistical analysis. Mean and SD were calculated for each variable for each group of lambs. The results in hypoxemic and control lambs were compared by means of an unpaired *t* test. Multiple linear regression was used to determine whether indices of oxygen demand, oxygen uptake, and supply were linearly related, and whether arterial substrate concentrations and left ventricular substrate uptake were linearly related. The effect of hypoxemia was evaluated by introducing a dummy variable for the experimental animal (28). Significance level was 0.05 for all comparisons.

RESULTS

General (Table 1). Hypoxemic lambs were slightly younger than control lambs and their body mass was lower. The arterial oxygen saturation was decreased and the Hb concentration was increased in hypoxemic lambs, so the arterial oxygen concentration was similar to that in control lambs. Heart rate was higher in hypoxemic lambs, but their systemic blood flow was not significantly increased. Although oxygen uptake was increased in hypoxemic lambs, systemic oxygen supply was similar to that in control lambs. Blood pressures were similar in hypoxemic and control lambs. Similarly, we found no difference in epinephrine and norepinephrine concentrations between the two groups of lambs. Hypoxemic lambs had a respiratory compensated metabolic acidosis.

Table 1. *Cardiovascular variables, systemic oxygen supply and oxygen uptake, pH and blood gases, and catecholamine concentrations*

	Control ($n = 14$)	Hypoxemia ($n = 15$)
Age (d)	46 ± 5	41 ± 6*
Body mass (kg)	12.5 ± 2.2	10.6 ± 2.3*
Oxygen saturation (%)		
Arterial	91 ± 3	67 ± 8*
Mixed venous	55 ± 5	39 ± 10*
Hb concentration ($g \cdot L^{-1}$)	102 ± 11	139 ± 16*
Arterial oxygen concentration ($mL \cdot L^{-1}$)	127 ± 22	129 ± 15
Oxygen uptake ($mL \cdot min^{-1} \cdot kg^{-1}$)	7.0 ± 1.9	8.3 ± 2.2*
Systemic blood flow ($mL \cdot min^{-1} \cdot kg^{-1}$)	145 ± 49	182 ± 73
Heart rate ($beats \cdot min^{-1}$)	118 ± 25	166 ± 33*
Systemic oxygen supply ($mL \cdot min^{-1} \cdot kg^{-1}$)	19 ± 5	23 ± 12
Mean blood pressure (kPa)		
Arterial	9.9 ± 1.0	10.1 ± 1.4
Right atrial	0.5 ± 0.4	0.9 ± 0.7
Arterial blood gases and pH		
pH	7.43 ± 0.04	7.39 ± 0.04*
P_{CO_2} (kPa)	5.2 ± 0.6	4.3 ± 0.7*
P_{O_2} (kPa)	13.3 ± 1.8	7.9 ± 1.3*
Bicarbonate ($mmol \cdot L^{-1}$)	24.5 ± 2.9	18.5 ± 3.6*
Epinephrine ($nmol \cdot L^{-1}$)†	1.2 ± 0.7	2.0 ± 3.8
Norepinephrine ($nmol \cdot L^{-1}$)†	10.9 ± 9.5	17.9 ± 26.8

* $p < 0.05$.
† $n = 13$ for control lambs; $n = 11$ for hypoxemic lambs.

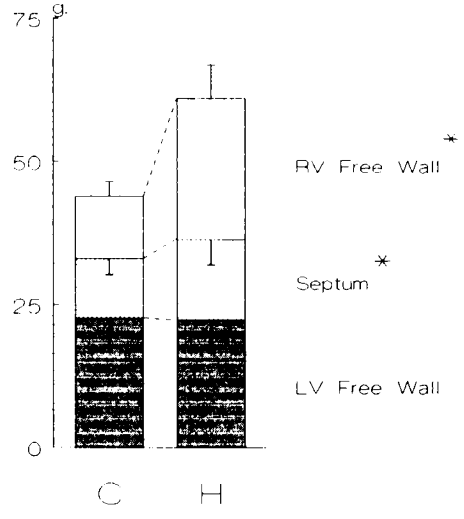

Fig. 1. Myocardial mass and its subdivision into left and right ventricular (*LV* and *RV*) free wall and septum in control lambs (*C*) and in lambs after 4 wk of hypoxemia (*H*). Mean and SD for each ventricular part are shown. *, $p < 0.05$.

Heart. Myocardial mass was increased in hypoxemic lambs, mainly because of an increased right ventricular mass (Fig. 1). Total myocardial blood flow was increased in hypoxemic lambs (102 ± 30 *versus* 56 ± 24 $mL \cdot min^{-1}$, $p < 0.001$). Per 100 g of tissue, blood flows to the left ventricle ($p < 0.05$), the septum (p

< 0.05), and the right ventricle ($p < 0.001$) all were significantly increased in hypoxemic lambs (Fig. 2). Left ventricular resistance was similar in hypoxemic and control lambs (0.06 ± 0.02 *versus* 0.08 ± 0.02 kPa·min·100 g·mL^{-1}). The coronary sinus blood in hypoxemic lambs had a lower oxygen saturation (16 ± 6 *versus* 25 ± 10%, $p < 0.01$) and Po_2 (3.7 ± 1.1 *versus* 4.6 ± 0.8 kPa, $p < 0.05$) than in control lambs. Consequently, the LVAVD for oxygen was maintained at similar levels in hypoxemic and control lambs (4.3 ± 0.8 *versus* 4.1 ± 0.8 mmol·L^{-1}).

Left ventricular oxygen supply and oxygen uptake were increased in hypoxemic lambs, but left ventricular oxygen extraction was similar in hypoxemic and control lambs (Fig. 3). Left ventricular oxygen uptake per beat was similar in hypoxemic and control lambs (4.5 ± 1.9 *versus* 4.5 ± 1.0 μmol·100 g^{-1}). The rate pressure product was increased in hypoxemic lambs (2072 ± 465 *versus* 1467 ± 358 kPa·beat·min^{-1}, $p < 0.001$). There was a linear relation between the rate pressure product and the left ventricular oxygen uptake ($y = 0.26x + 173$, $r = 0.59$, $p < 0.001$), and hypoxemia had no effect on this relationship. In contrast, stroke work was similar in hypoxemic and control lambs (158 ± 88 *versus* 189 ± 77 kPa·mL), and no relation between stroke work and left ventricular oxygen uptake per beat was found. Left ventricular oxygen supply increased linearly with oxygen uptake (Fig. 4), and hypoxemia had no effect on this relationship.

Arterial pyruvate, lactate, and β-hydroxybutyrate concentrations were slightly, but significantly, increased in hypoxemic lambs, whereas the other substrate concentrations were similar to that in control lambs (Fig. 5). In addition, the lactate/pyruvate ratio was similar in hypoxemic and control lambs (18.6 ± 8.5 *versus* 22.7 ± 13.1). Left ventricular uptake of pyruvate and acetoacetate was slightly different in hypoxemic lambs compared with control lambs, whereas for the other substrates no differences were found (Fig. 5); there was no net lactate production. The OER, however, indicate that the contribution of pyruvate and acetoacetate to left ventricular substrate uptake was only small (Table 2). FFA and β-hydroxybutyrate were the most important fuels for the left ventricle that we identified, whereas the contribution of glucose, pyruvate, and lactate was small in both hypoxemic and control lambs (Table 2). Left ventricular uptake of ketoacids increased with increasing arterial substrate concentration (β-hydroxybutyrate: $y = 0.05x - 0.4$, $r = 0.58$, $p < 0.001$; acetoacetate: $y = 0.04x - 1.4$, $r = 0.63$, $p < 0.001$), whereas for the other substrates such a relation was not found. The OER of all substrates combined adds up to only 0.5, indicating that we identified approximately 50% of the fuels for the left ventricle.

DISCUSSION

In the present study we demonstrated that, in lambs after 4 wk of hypoxemia, left ventricular oxygen supply and oxygen uptake are proportionally increased to meet the increased oxygen demand of the left ventricle. The increase in oxygen demand and oxygen uptake is directly related to the increased heart rate in hypoxemic lambs. We also demonstrated that left ventricular substrate uptake is practically unaltered in hypoxemic lambs compared with control lambs. Despite the increased arterial concentrations of lactate, pyruvate, and β-hydroxybutyrate in hypoxemic lambs, the uptake of these substrates by the left ventricle is not increased. In addition, glucose, pyruvate, and lactate uptake is negligible, and FFA and ketoacids only supply approximately 50% of the fuel for the left ventricle in the lambs in our study.

Left ventricular blood flow is high after birth (200 mL·min^{-1}·100 g^{-1}), but gradually decreases to approximately 50% of this value in adult sheep (29, 30). This is related to a decrease in left ventricular oxygen uptake per unit mass and an increase in Hb concentration (26, 29, 30). The increase of the left ventricular blood flow, in our hypoxemic lambs, was not needed to compensate for the decreased arterial oxygen saturation, because the arterial oxygen concentration was similar to that in control lambs. Instead, the increased left ventricular blood flow maintained the left ventricular oxygen supply matched to oxygen uptake (Fig. 4). It is readily apparent from Figure 4 that the matching of left ventricular oxygen supply to oxygen uptake is unaltered in hypoxemic lambs compared with control lambs. The increase of the left ventricular oxygen uptake in hypoxemic lambs was related to their increased heart rate, because per beat both oxygen demand, as estimated by stroke work, and oxygen uptake were similar in hypoxemic and control lambs. Right ventricular blood flow was also increased in hypoxemic lambs, presumably to match right ventricular oxygen supply to an increased right ventricular oxygen demand. The increased heart rate will affect right ventricular oxygen demand in a fashion similar to left ventricular oxygen demand. In addition, the work load imposed on the right ventricle in hypoxemic lambs most likely is increased because of the pulmonary artery banding (31). However, the increase in right ventricular oxygen supply, in these conditions, may be out of proportion to the increase in oxygen demand (31). Thus, the 2-fold increase in blood flow to the ventricular myocardium in hypoxemic lambs can be ascribed in part to the effects of the increased heart rate and, presumably, to the effects of the pulmonary artery banding on right ventricular oxygen demand and supply.

An alteration in myocardial blood flow is the most important mechanism to maintain an adequate myocardial oxygen supply

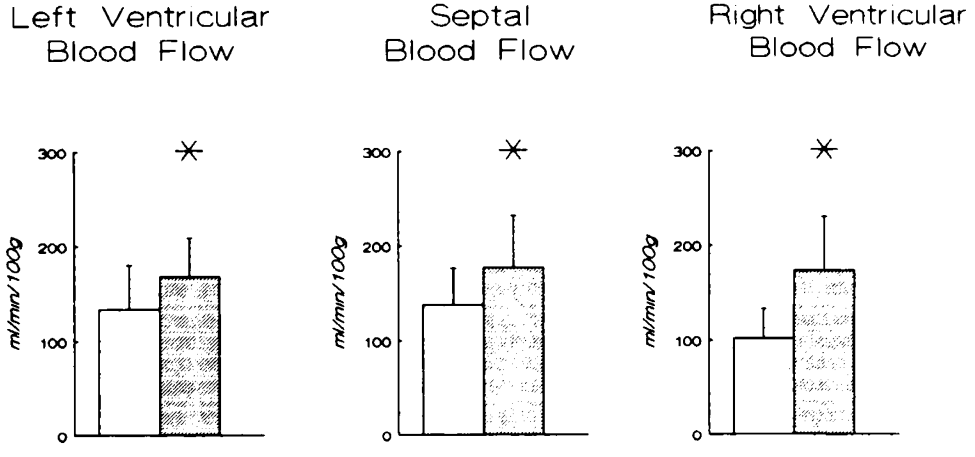

Fig. 2. Blood flow to the ventricular myocardium per 100 g in control lambs (*open bars*) and in lambs after 4 wk of hypoxemia (*hatched bars*). *, $p < 0.05$.

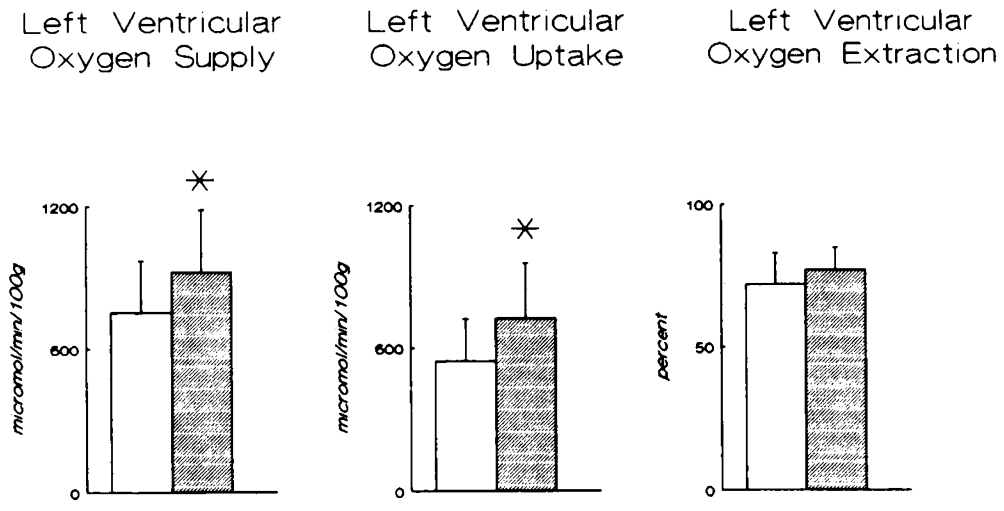

Fig. 3. Left ventricular oxygen supply, oxygen uptake, and oxygen extraction in control lambs (*open bars*) and in lambs after 4 wk of hypoxemia (*hatched bars*). *, $p < 0.05$.

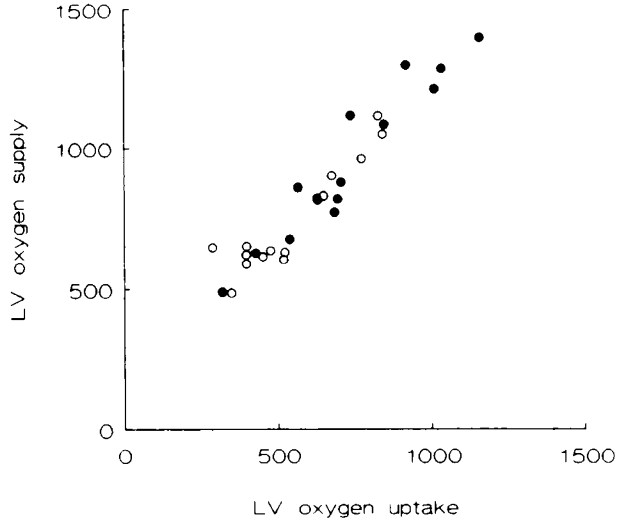

Fig. 4. Left ventricular (*LV*) oxygen uptake ($\mu mol \cdot min^{-1} \cdot 100\ g^{-1}$) *vs* left ventricular oxygen supply ($\mu mol \cdot min^{-1} \cdot 100\ g^{-1}$) in control lambs (*open circles*) and in hypoxemic lambs (*black circles*). Regression for all animals: $y = 1.08x + 158$, $r = 0.96$, $p < 0.001$.

under various pathophysiologic conditions, because the extraction reserve of the heart is limited. Myocardial blood flow is increased to increase oxygen supply in proportion to oxygen uptake (32) or to maintain oxygen supply during (severe) acute hypoxemia or anemia (1–3, 5, 6). Conversely, myocardial blood flow decreases during acute polycythemia (1, 3, 5). These adjustments in myocardial blood flow are the result of alterations in vascular tone. In case of acute hypoxemia, the change in resistance reflects the change in vascular tone. However, in case of anemia or polycythemia, a change in resistance reflects the combined result of the alteration in vascular tone and the alteration in whole blood viscosity. For example, in polycythemic dogs coronary resistance increased, but coronary vascular tone decreased to maintain myocardial oxygen supply (3). Similarly, maximal myocardial oxygen supply decreased during progressive polycythemia in dogs, indicating that the viscosity of blood increased relatively more than the oxygen capacity (1). In another set of experiments, we found that whole blood viscosity in hypoxemic lambs was increased compared with control lambs (4.4 *versus* 3.6 mPa·s, shear rate 100 s^{-1}, unpublished observations). The difference in vascular tone in these conditions can be estimated by the ratio of resistance and whole blood viscosity (3). Because left ventricular resistance in hypoxemic lambs was

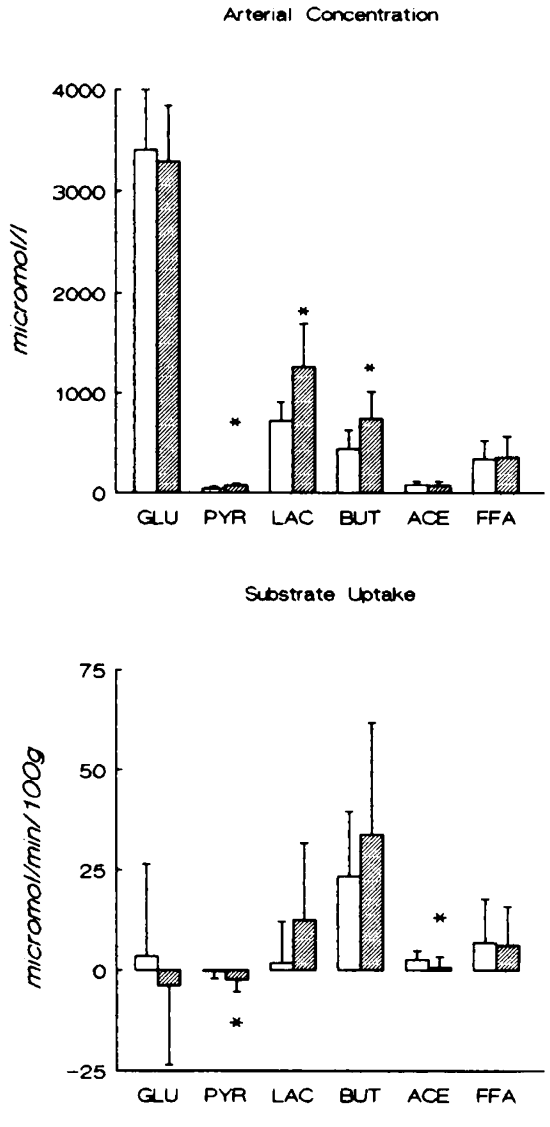

Fig. 5. Arterial substrate concentration and substrate uptake in control lambs (*open bars*) and in lambs after 4 wk of hypoxemia (*hatched bars*). *GLU*, glucose; *PYR*, pyruvate; *LAC*, lactate; *BUT*, β-hydroxybutyrate; *ACE*, acetoacetate. *, $p < 0.05$.

Table 2. *OER*

	Control ($n = 14$)	Hypoxemia ($n = 15$)
Glucose	0.02 ± 0.22	−0.04 ± 0.20
Pyruvate	0.00 ± 0.01	−0.01 ± 0.01
Lactate	0.01 ± 0.05	0.05 ± 0.08
β-Hydroxybutyrate	0.18 ± 0.09	0.20 ± 0.11
Acetoacetate	0.02 ± 0.02	0.00 ± 0.02
FFA*	0.28 ± 0.38	0.19 ± 0.32
Total*	0.51 ± 0.50	0.45 ± 0.43

* $n = 11$ for hypoxemic lambs.

approximately 0.8 of that in control lambs and whole blood viscosity 1.25 of that in control lambs, this ratio in hypoxemic lambs was approximately 0.7 (0.8/1.25) of that in control lambs, indicating a decreased vascular tone in the left ventricular myocardium in hypoxemic lambs, and consequently vasodilation. This is corroborated by the observation that, in our hypoxemic lambs, both the oxygen tension and the oxygen saturation of the coronary sinus blood were decreased.

An increase in myocardial blood flow is established at the expense of the coronary flow reserve, unless the microvascular bed has been increased through capillary proliferation. In chronically hypoxemic animals, an increased capillary density has been found in skeletal muscle, brain, and heart (33, 34). In all studies, an increased capillary density is found in the right ventricle (34–37). However, conflicting results have been reported about the left ventricular capillary density in chronic hypoxemia. In some studies, an increased capillary density of the left ventricle has been found (34), whereas in other studies no effect of hypoxemia on the capillary density of the left ventricle was observed (35–37). Because left ventricular capillary density may not be increased in hypoxemic lambs, the increase in flow rate in hypoxemic lambs may be established at the expense of coronary flow reserve.

Arterial lactate and pyruvate concentrations were slightly increased in hypoxemic lambs, which cannot be explained by an increased catecholamine concentration (Table 1). Because the lactate/pyruvate ratio was not increased in hypoxemic lambs, the higher lactate concentration may be related to decreased utilization rather than to increased anaerobic glycolysis. In children with cyanotic heart disease, lactate and pyruvate concentrations were increased after fasting compared with those in noncyanotic subjects (38), possibly through a decreased hepatic uptake of lactate and pyruvate secondary to decreased hepatic perfusion. A decreased hepatic blood flow has indeed been found in chronically hypoxemic lambs (10).

Left ventricular substrate uptake in hypoxemic lambs was practically unaltered compared with that in control lambs, and FFA and β-hydroxybutyrate were important fuels, whereas the uptake of glucose, pyruvate, and lactate was negligible. Similar results have been obtained in 6-wk-old lambs with and without an aortopulmonary left-to-right shunt in our laboratory (12). A low myocardial glucose, pyruvate, and lactate uptake has also been found in newborn lambs and adult sheep (29, 30). In contrast, myocardial lactate and pyruvate uptake increased linearly with arterial substrate concentration in chronically hypoxemic adults, native to high altitude (20). In that study, myocardial oxygen uptake was decreased, which was related to an increased efficiency (9). It was postulated that some adaptation of cellular metabolism might be responsible for these findings. If so, these may take longer to develop than the duration of hypoxemia in our lambs.

The total oxygen extraction ratio was approximately 0.5 in both groups of lambs, indicating that we identified only 50% of the fuels for left ventricular oxidative metabolism. This may result from the utilization of substrate from endogenous stores or the uptake of substrate that we did not measure. The predominant uptake of fatty acids and ketoacids and the low uptake of glucose and pyruvate in our lambs is compatible with a postabsorptive state (39). In these conditions, the breakdown of glycogen as well as the utilization of glucose through the glycolytic pathway is inhibited (40), so that glucose utilization from endogenous stores is unlikely. The triglyceride pool is another source of endogenous substrate. Zierler (41) suggested that all fatty acids taken up by the myocardium are initially stored in a pool and are used from this pool. In fasted, healthy human subjects, approximately 85% of the ^{14}C-labeled palmitate or oleate taken up by the myocardium underwent oxidation within 30 min (42). In anesthetized dogs, virtually all the ^{14}C-labeled palmitate taken up by the myocardium underwent rapid oxidation (43), and the oxidation of glucose, lactate, and fatty acid uptake accounted for all the oxidative metabolism in resting conditions (42, 44). These results suggest that even if all FFA taken up by the myocardium are initially stored in a pool, their use from this pool is rapid. Thus, the low OER in our lambs cannot readily be explained by the utilization of substrates from endogenous stores.

Substantial uptake of substrates that we did not measure will also lead to an OER lower than 1. In another study from our laboratory, we demonstrated that triglycerides also contributed to left ventricular substrate uptake (12). In addition, in sheep large amounts of short-chain fatty acids are produced in the rumen, and acetate is largely delivered to peripheral tissues (45). In resting skeletal muscle, oxidation of acetate accounted for ≈20% of the oxygen uptake, whereas during exercise this decreased to ≈5% (46). Thus, acetate may be an important additional fuel for myocardial oxidative metabolism in lambs.

In summary, we have demonstrated that, in chronically hypoxemic lambs, left ventricular oxygen supply is matched to an increased oxygen demand by adjustments in left ventricular blood flow. The relation between left ventricular oxygen supply and oxygen uptake is unaltered in chronic hypoxemia compared with normoxemia. Left ventricular substrate uptake in chronically hypoxemic lambs is practically unaltered compared with control lambs. We identified that FFA and β-hydroxybutyrate are the most important substrates taken up by the myocardium, but we speculate that triglycerides and acetate may also contribute to left ventricular substrate uptake.

Acknowledgments. The authors thank Ineke Top-Dalebout and Marchien Velvis (Central Laboratory for Clinical Chemistry, University Hospital, Groningen) for measuring FFA and catecholamine concentrations, and Ineke Wessels for assistance in preparing the manuscript.

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