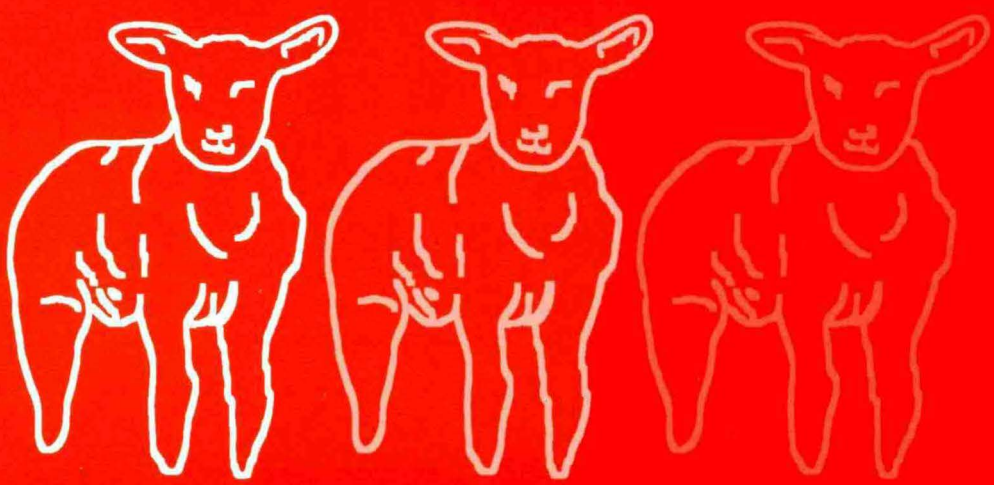
Chronic Hypoxemia in Lambs with Experimental Cyanotic Heart Disease

Cardiovascular, hematological, and metabolic adjustments, and growth



Michiel Dalinghaus

Chronic Hypoxemia in Lambs with Experimental Cyanotic Heart Disease

Stellingen behorend bij het proefschrift

Chronic hypoxemia in lambs

with

experimental cyanotic heart disease

Michiel Dalinghaus

Groningen, 19 oktober 1994

1. Chronische hypoxemie gaat zelden gepaard met hypoxie.

2. De toename van de hemoglobineconcentratie bij chronische hypoxemie is geen adaptatie.

3. Een matige toename van de viscositeit van het bloed, die optreedt als gevolg van de aanpassing aan chronische hypoxemie, heeft een klinisch relevant effect op de doorstroming van organen (dit proefschrift).

4. Het beperkte vermogen van de foetale linker ventrikel om het minuutvolume te vergroten wordt niet verklaard door "onrijpheid" van de hartspier.

5. Gluconeogenese speelt geen rol van betekenis bij het handhaven van de bloedsuikerspiegel voor de geboorte.

6. De onderhoudsbehandeling van decompensatio cordis (circulatoire congestie) op de kinderleeftijd met behulp van lis-diuretica is niet bewezen effectiever dan de behandeling met thiazide-diuretica.

7. Een kind met acute benauwdheid dient in consult te worden *gezien*.

8. Bij de behandeling van een pasgeborene met hyperbilirubinemie dient behalve met de "geelheid" van de baby eveneens rekening te worden gehouden met de "blues" van de ouders.

9. Op grond van goed uitgevoerd onderzoek is de causale relatie tussen de inname van bepaalde voedingsbestanddelen, zoals suikers en kleurstoffen, en gedragsproblemen niet aangetoond. Indien bij patienten met gedragsproblemen een dergelijk verband uitgesloten moet worden, dient het veronderstelde agens (dubbel-)blind en placebo-gecontroleerd aangeboden te worden.

10. Teneinde papierverspilling terug te dringen, dient bij de overdracht van (voorlopige) informatie binnen geautomatiseerde systemen, het gebruik en het gebruiksgemak van beeldschermen krachtig gestimuleerd te worden.

11. De opleiding tot medisch specialist blijkt als een effectief, maar selectief, contraceptivum te werken. Om aan deze ongewenste bijwerking een einde te maken, dient de status van de ZwAGIO formeel geregeld te worden.

12. Om ergens als de kippen bij te kunnen zijn is haantjesgedrag vaak noodzakelijk.

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door

Michiel Dalinghaus
geboren op 10 mei 1957
te Vlissingen

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voor Els,
Francien en Nienke

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Dankwoord - acknowledgements

Nu dit boekje bijna in het bakje "afgewerkt" gelegd kan worden, is het me een genoegen om allen die aan de totstandkoming ervan een bijdrage hebben geleverd te bedanken voor de plezierige samenwerking, de gezelligheid en de opbeurende schouderklop van tijd tot tijd. Zonder iemand tekort te willen doen, zou ik de volgende personen in het bijzonder willen bedanken.

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1

Introduction

Congenital cyanotic heart disease is one of the most important causes of chronic hypoxemia in infancy and childhood. Congenital heart disease affects 8-10 out of 1000 live-born children, and 15-20 % of these children have cyanotic heart disease [109, 226]. The severity of the hypoxemia is quite variable, ranging from barely detectable on physical examination to severe hypoxemia. In the most severe forms of hypoxemia early and vigorous medical therapy and surgical intervention may be required. However, most infants have mild to moderate hypoxemia and do not require early intervention to survive. In the majority of cases corrective surgery soon after birth is not feasible, so that most infants born with cyanotic heart disease will have to adjust to some degree of chronic hypoxemia.

Perinatal changes in gas exchange and circulation

Before birth gas, exchange takes place in the placenta and the arrangement of the fetal circulation is such that the relatively well oxygenated venous return from the placenta is preferentially distributed towards the left ventricle. It is ejected into the ascending aorta, mainly supplying blood to the upper body, including the brain and the heart [184]. The relatively deoxygenated venous return from the body is preferentially distributed towards the right ventricle. It is ejected into the main pulmonary artery, but largely shunted away from the pulmonary circulation through the ductus arteriosus into the descending aorta, mainly supplying blood to the lower body, including the umbilical circulation [184]. After birth, gas exchange takes place in the lungs. The arrangement of the postnatal circulation is such that the deoxygenated venous return from the body goes to the right ventricle, which supplies it to the pulmonary circulation. The oxygenated venous return from the lungs goes to the left ventricle, which supplies it to the systemic circulation.

All forms of congenital cyanotic heart disease have in common that part of the deoxygenated venous return from the body does not pass the pulmonary circulation, but directly re-enters the systemic circulation. The degree of arterial hypoxemia depends on the flow ratio of oxygenated and deoxygenated blood. Because in the fetus the pulmonary blood flow is low and the lungs do not contribute to gas exchange, most forms of congenital cyanotic heart disease have no appreciable effect on the oxygen supply to the fetus. For this reason most live-born infants with congenital cyanotic heart disease have a normal growth and development for gestational age and show no signs of adjustment to chronic hypoxemia at birth.

Oxygen transport in cyanotic heart disease

Oxygen transport from outside air to the interior of the cells in the body can be divided into 4 phases. First, convection of air to the alveoli driven by the ventilatory

pump. Second, diffusion of oxygen from the alveoli to the blood in lung capillaries. Third, convection of oxygen by the blood from the lungs to the tissues driven by the circulatory pump and, fourth, diffusion of oxygen from the tissue capillaries to the cells. When the oxygen transport system is disrupted, adjustments in any of the four phases of oxygen transport may be helpful in maintaining an adequate oxygen supply of the tissues. In congenital cyanotic heart disease the first and second phase of oxygen transport are generally unimpaired and adjustments in these phases will hardly improve oxygen supply to the tissues. Increasing the ventilation will increase the alveolar and the pulmonary capillary oxygen tension, but it will hardly affect the oxygenation of hemoglobin, because of the sigmoid shape of the oxygen dissociation curve. Diffusion of oxygen from the alveoli to the pulmonary capillaries is generally not impaired, especially not in those forms of congenital heart disease with a decreased pulmonary blood flow, like tetralogy of Fallot. In contrast, in the third and fourth phases of oxygen transport a number of adjustments are possible that will improve the oxygen supply to the tissues. In this thesis we will focus on these adjustments to chronic hypoxemia and their consequences.

Systemic oxygen supply is determined by systemic blood flow (Q_S), the hemoglobin concentration ([Hb]), the oxygen binding capacity of hemoglobin (β), and the fractional arterial oxygen saturation (S_{aO_2}):

$$\text{Systemic oxygen supply} = Q_S \times \beta \times [Hb] \times S_{aO_2}$$

When the arterial oxygen saturation is decreased, both an increase in systemic blood flow and an increase in hemoglobin concentration may restore systemic oxygen supply. The oxygen binding capacity of hemoglobin is a constant. Increasing the systemic blood flow is an instantaneous adjustment that is found in acute hypoxemia [1, 201], but systemic blood flow returns to prehypoxemic values after prolonged exposure to hypoxemia [127, 134, 213]. Therefore, the maintenance of systemic oxygen supply in chronic hypoxemia is generally not related to an increase in systemic blood flow. The increase of the hemoglobin concentration, which increases the oxygen capacity of the blood, is a much slower adjustment to hypoxemia, that develops over days to weeks. After prolonged exposure to hypoxemia an increase in hemoglobin concentration is an almost uniform response. Therefore, the maintenance of systemic oxygen supply in chronic hypoxemia is largely related to the increase in the oxygen capacity of the blood. In the last phase of oxygen transport, the diffusion of oxygen from the tissue capillaries to the cell, several adjustments may improve oxygen transport. First, a decrease in the oxygen affinity of hemoglobin will allow more oxygen to be unloaded for any given oxygen tension. Second, an increased capillary density, brought about either by vasodilation or, structurally, by the formation of new capillaries, will decrease the

diffusion distance from the capillaries to the cells and will allow oxygen to diffuse at a lower capillary oxygen tension.

The adjustments to chronic hypoxemia that are found in children with congenital cyanotic heart disease are in accordance with the above considerations. Systemic blood flow is maintained, whereas the hemoglobin concentration is increased [26]. In some cases the oxygen affinity of hemoglobin is decreased, but this is not a uniform finding [26, 231]. In part this non-uniformity of the decrease in oxygen affinity may be related to the presence or absence of fetal hemoglobin. The interpretation of the results of clinical studies is complicated by the heterogeneity of the groups of patients with respect to the severity of their cyanotic heart disease and their differences in age. Therefore, the relative contributions of changes in oxygen capacity and affinity remain uncertain.

One of the potential disadvantages of the increased oxygen capacity of the blood is the effect of the concomitant rise in whole blood viscosity. Even under normoxemic conditions a negative effect of a high hemoglobin concentration (high hematocrit) on systemic oxygen supply can be demonstrated though the arterial oxygen concentration is increased, because at high hematocrit the whole blood viscosity increases more than the oxygen capacity of the blood [46, 65]. The perfusion of organs is impaired in polycythemic conditions [65] and even the perfusion and thus the oxygen supply of heart and brain may be impaired under certain circumstances [6, 147]. In chronic hypoxemia the increased hemoglobin concentration serves as a compensation for the decreased arterial oxygen saturation, so that the arterial oxygen concentration is generally normalized. Therefore, the effects of an increased whole blood viscosity on oxygen supply to the tissues may be expected sooner in chronic hypoxemia than in normoxemic polycythemia. At very high hematocrit the disadvantages of the increased whole blood viscosity have been clearly demonstrated [185, 215]. However, the effects of a moderate increase of the whole blood viscosity on organ blood flow and oxygen supply in chronic hypoxemia are not well known.

Myocardial oxygen supply and uptake in cyanotic heart disease

Some investigators have suggested that myocardial function is impaired in congenital cyanotic heart disease [86, 233]. For this several explanations have been proposed, including an impairment of myocardial oxygen supply [125, 204]. Because the myocardium has a limited oxygen extraction reserve, an adequate myocardial oxygen supply is closely linked to an adequate myocardial blood flow. Studies in chronically hypoxemic subjects, native to or sojourning at high altitude, have demonstrated that myocardial blood flow and oxygen supply were decreased [93, 159] and that myocardial oxygen uptake was decreased [159] or maintained by an increase in oxygen extraction [93]. In contrast to these observations, other

investigators have demonstrated that myocardial function was not impaired at high altitude [94, 177], suggesting that hypoxemia per se may not cause an impairment of myocardial function. In addition, a decrease of the myocardial blood flow and the consequent decrease of myocardial oxygen supply does not seem a logical adjustment at moderate high altitude. Although the polycythemia that develops during chronic hypoxemia may impair myocardial blood flow, because of the consequent increase in whole blood viscosity, the coronary flow reserve seems to be sufficient to allow myocardial blood flow to increase in order to meet myocardial oxygen demand [6]. In acute hypoxemia myocardial blood flow can be increased to such an extent, that myocardial oxygen supply is maintained even at very low arterial oxygen saturations [70, 75, 170, 201, 202]. It is not known, however, whether myocardial oxygen supply is adequately matched to oxygen demand in congenital cyanotic heart disease in steady state hypoxemia, or when acute hypoxemia is superimposed on chronic hypoxemia.

Growth

Growth disturbance has been demonstrated in children with congenital heart disease [83, 135, 150]. In most cases the decreased growth rate is related to defects that are accompanied by congestive heart failure. In these conditions a decreased energy intake and/or increased energy expenditure may explain the growth failure. In congenital cyanotic heart disease with a decreased pulmonary blood flow, like tetralogy of Fallot, congestive heart failure is almost never found, but growth failure has been demonstrated in these patients [83]. This indicates that hypoxemia per se may affect growth, which is also suggested by studies in chronic hypoxemia induced by (simulated) high altitude [163, 221, 244] or experimental cyanotic heart disease [213]. The mechanism of growth failure in chronic hypoxemia has not been elucidated, nor has it been established how the growth of organs, the development of lean body mass, and its constituents are affected during the early phase of adjustment to chronic hypoxemia.

Responses to stress

Induction of acute hypoxemia evokes responses that serve to maintain an adequate oxygen supply to the tissues. Heart rate and systemic blood flow increase in order to maintain systemic oxygen supply as high as possible. In addition, blood flow and oxygen supply are redistributed in order to meet the oxygen demands of those tissues that are crucial to immediate survival. These adjustments are mediated through neurohumoral and chemoreceptor responses [106]. During prolonged hypoxemia most of the adjustments to acute hypoxemia abate and are in part replaced by adjustments that develop more slowly, like the increased hemoglobin concentration. After prolonged exposure to hypoxemia, the ability to respond to a further reduction in the arterial oxygen saturation may be blunted. Episodes of an

acute reduction of the arterial oxygen saturation may occur in patients with congenital cyanotic heart disease during so-called hypoxic spells. It is unknown whether these patients are capable of an adequate cardiovascular response to acute hypoxemia that is superimposed on chronic hypoxemia. The development of models of experimental cyanotic heart disease allows studies that increase our insight into the adjustments to acute hypoxemia in these conditions.

Recent research

In 1985 Teitel and coworkers described a model of experimental cyanotic heart disease, obtained by creating an atrial septal defect in combination with a variable pulmonary stenosis in lambs [213]. Since then several papers have been published by Teitel and coworkers, Bernstein and coworkers, and from our laboratory, addressing various aspects of the adjustments to chronic hypoxemia. In these studies cardiovascular and hematological adjustments [31, 32, 49, 50], some aspects of the mechanism of growth retardation in chronic hypoxemia [27, 29, 48], and responses to stress [28] were described. The similarities between the various measurements demonstrate how well the data obtained by the three laboratories can be compared, while the diversity of the issues addressed increases our insight into the pathophysiology of the (early) adjustments to congenital cyanotic heart disease.

Aim and scope of the study

In this thesis we describe studies on cardiovascular, hematological, metabolic, and growth adjustments to chronic hypoxemia in lambs with experimental cyanotic heart disease. It was our purpose to determine the factors that help to maintain oxygen supply to and oxygen uptake by the tissues in chronic hypoxemia. In addition, we wanted to determine the effects of chronic hypoxemia on growth. Finally, we wanted to determine the adequacy of the cardiovascular response to acutely induced stress.

In order to reach our aims, we studied the contributions of cardiovascular adjustments, and of changes in oxygen capacity and oxygen affinity towards maintaining systemic oxygen supply and oxygen uptake (Chapter 3). The effect of the increased whole blood viscosity, as a consequence of the increased hematocrit, on organ blood flow and oxygen supply was studied by determining organ blood flows in relation to the whole blood viscosity (Chapter 4). Specifically, the effects of hypoxemia on myocardial blood flow and on the relation between left ventricular oxygen demand, supply, and uptake were determined (Chapter 6). The effects of hypoxemia on the development of (lean) body mass and its compartments were studied by measuring body fluid compartments (Chapter 5). This also allowed us to study the effects of hypoxemia on blood volume and total red cell mass. Finally, we determined the adequacy of the cardiovascular response to acutely induced

hypoxemia superimposed on chronic hypoxemia, in order to test the ability to adjust to hypoxic spells in cyanotic heart disease (Chapter 7).

2

Materials and methods

In the experiments described in this thesis we studied chronic hypoxemia in lambs, induced by the combination of a variable pulmonary stenosis and an atrial septal defect, which was created by performing a (Rashkind) balloon septostomy. All the hypoxemic lambs had undergone surgery before the tenth day of life, usually in the first week. The lambs were allowed to adapt to hypoxemia for 3-4 weeks before the measurements were made. Twenty-one hypoxemic lambs were studied, 4 were used in four protocols, 5 were used in three protocols, 2 were used in two protocols, and 10 were used in only one protocol. There were several reasons why the hypoxemic lambs could not be used in all experimental protocols. Mortality rate was high, the main identifiable cause of death being pulmonary rupture due to pressure necrosis of the main pulmonary artery. Catheter dysfunction was another reason not to use lambs in certain protocols. Some of the lambs were also used in studies not described in this thesis.

Six control lambs underwent surgery before the 10th day of life and were used in the protocol described in Chapter 3. Nineteen control lambs underwent surgery at least one week before the measurements; this period is sufficient to completely recover from surgery [200]. In addition, 11 control lambs underwent neck vessel catheterization at least 24 h before the measurements. One control lamb was used in three protocols, 10 were used in two protocols and 25 were used in only one protocol. Most control lambs participated in other experimental protocols as well.

All lambs were allowed at least 48 h of recovery between two studies. Each lamb remained with its mother throughout the period it was in the laboratory.

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). Inspiratory volume was 15-20 mL/kg and ventilation frequency 15-20 min^{-1}. Analgesia was maintained with piritramide 10-20 mg intramuscularly and lidocaine hydrochloride (5 g/L) 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 hemiazygous vein was ligated 1-3 cm from its entrance into the pericardium and a catheter was advanced towards the heart with its tip placed at the confluence with the coronary sinus. Subsequently, the pericardial sac was carefully opened, taking care not to damage the phrenic and vagal nerves, 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 5F balloon-tipped Fogarty catheter (American Edwards Laboratories, Santa Ana, CA, USA), which was introduced through a pedal vein. After positioning the tip in 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. An inflatable silicone rubber constrictor, inner diameter 8-10 mm, (Hazen Everett Co., Teaneck, NJ, USA) was fitted around the main pulmonary artery. An 8F 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. Control lambs underwent the same surgical procedure apart from the atrial septostomy, the placement of the constrictor around the pulmonary artery and the insertion of a right ventricular catheter. The left thoracic cavity was aspirated daily for 4-6 days, then the thoracic drain was removed.

Neck vessels were catheterized by incising the skin over the right carotid artery and jugular vein under local anesthesia by lidocaine hydrochloride (5 g/L). A balloon-tipped catheter, that was connected to a pressure transducer, was inserted into the jugular vein and positioned in the pulmonary artery, while the pressure tracing was monitored. The right carotid artery was ligated and a catheter was advanced into the ascending aorta.

All lambs were given 150 mg ampicillin intramuscularly for 4 days after surgical procedures. Daily, all lambs were weighed and the catheters refilled with fresh heparin solution. Weekly, each lamb was given iron dextran complex intramuscularly equivalent to 200 mg iron.

Induction of hypoxemia was started three to five days after surgery by inflating the constrictor around the pulmonary artery with sterile saline solution (9 g/L), thus inducing a right-to-left shunt through the atrial septal defect (Fig 2.1). 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% and to keep it within this range.

Experimental protocols

All measurements were made in a room with a temperature of 19-23°C. During the measurements the lamb was quietly resting in a canvas sling and supported in the upright position. Body temperature was monitored by a rectal temperature probe.

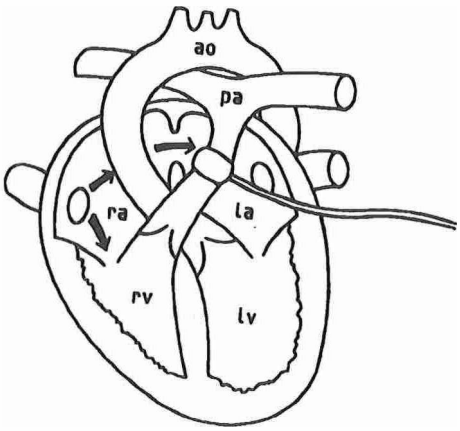

Figure 2.1 Diagram of the way the cardiac right-to-left was induced. After inflation of the constrictor around the pulmonary artery, a part of the systemic venous return shunted through the atrial septal defect, from the right atrium to the left atrium. pa=pulmonary artery, ao=aorta, rv=right ventricle, lv=left ventricle, ra=right atrium, la=left atrium. *Illustration by Jan H. Koers*

Cardiovascular and hematological variables, and oxygen uptake

These variables were obtained in all experiments, except in those concerning the measurement of body fluid compartment volumes (Chapter 5). Oxygen uptake was continuously recorded for 30 min. Blood pressures were measured every 5 min in aorta and left and right atrium. Blood samples were withdrawn, at 15 and 30 min, from the aorta and pulmonary artery or right ventricle to measure oxygen saturation, hemoglobin concentration, hematocrit, and blood gases.

Left ventricular oxygen and substrate uptake, and organ blood flows

Blood samples to measure oxygen (0.7 mL) and substrate concentrations (7 mL) were simultaneously withdrawn from the aorta and the coronary sinus, after the cardiovascular and hematological variables were obtained, or at the end of an intervention period. Immediately thereafter, organ blood flows were determined by injecting radiolabeled microspheres into the left atrium, while a reference sample was simultaneously withdrawn from the ascending aorta.

Body fluid compartment volumes

Plasma, extracellular water and total body water volumes were determined by means of a single-injection triple-indicator dilution technique. A freshly prepared mixture of indicators was injected into the pulmonary artery. Just before the injection (time zero), and at 3, 6, 9, 12, 15, 20, 25, 30, 40, 50, 60, 75, 90, 105, 120, 135, 150, 165 and 180 min after the injection, 2-4 mL blood samples were withdrawn from the aorta into tubes containing dried heparin. Hematocrit of the blood samples taken at 0, 20, 30, 40, 50 and 60 min was determined in duplicate. Before time zero, blood was obtained to measure plasma osmolality and total protein concentration. After the withdrawal of each blood sample, an equal volume of sterile saline (9 g/L) was injected into the pulmonary artery; a total volume of 60-70 mL blood was withdrawn during the experiment.

Induction of acute hypoxemia superimposed on chronic hypoxemia

After obtaining baseline cardiovascular and hematological data and determining organ blood flows by the microsphere method, the arterial oxygen saturation was acutely decreased by further inflating the constrictor around the pulmonary artery. We attempted to decrease the arterial oxygen saturation to approximately 50 % of its baseline value in each hypoxemic lamb. After 5-10 min of stabilization we obtained cardiovascular, hematological and oxygen uptake measurements for 15 min. At 10 and 15 min ascending aortic, mixed venous, and coronary sinus blood samples were withdrawn to measure oxygen saturation, hemoglobin concentration, hematocrit, blood gases and pH, and lactate concentration. Organ blood flows were determined by the microsphere method, as described above, at the end of the 15 min period.

Autopsy and dissection

At the end of all experiments, the lambs were killed by the injection of an overdose of pentobarbital. At autopsy all catheter positions were verified. Organs were taken out, stripped of peri-organ fat, and contents removed from hollow organs. Then organs were weighed. The brain was divided into left and right hemisphere and brain stem. The heart and the left kidney were stored in formalin (8 %) for 5-8 days. Thereafter, the heart was divided into atria, ventricular septum and left and right ventricular free walls. Each ventricular part was divided into an endocardial, middle and epicardial layer. The cortex of the left kidney was divided into an inner, middle and outer layer. The gastrointestinal tract was divided into esophagus, stomach, small intestine and large intestine. All organs or organ parts were then 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, Delft, The Netherlands). The lamb was breathing into a plastic bag and the mixture of expired air and room air was continuously withdrawn from the bag at a constant flow rate of 8 or 16 L/min and led through the Diaferometer. Blood pressures were measured by Gould P23 ID transducers (Spectramed Inc., Oxnard, CA, USA). The zero reference point was at mid-chest position. All pressure signals, phasic and mean, were recorded on an 8-channel Elema Mingograf recorder (Siemens-Elema AB, Solna, Sweden). Heart rate was calculated from the phasic aortic pressure tracing. Blood gases were measured in 0.8 mL samples by an ABL2 (Radiometer A/S, Copenhagen, Denmark) at 37°C and corrected to actual body temperature. Oxygen saturation, hemoglobin concentration, and hematocrit all were measured in duplicate: oxygen saturation by an OSM2 (Radiometer A/S, Copenhagen, Denmark), hemoglobin concentration by the cyanomethemoglobin method, hematocrit by the microhematocrit method. The oxygen concentration of the blood was calculated by using an oxygen-binding capacity of 1.36 mL/g [138]. The systemic blood flow was calculated by the Fick method, dividing the systemic oxygen uptake by the arterio-mixed venous oxygen concentration difference. Systemic oxygen supply was calculated as the product of arterial oxygen concentration and systemic blood flow. The oxygen extraction was calculated as the difference between arterial and mixed-venous oxygen saturation divided by the arterial oxygen saturation. The resistance in any vascular bed was calculated as the difference between mean arterial and right atrial pressure divided by the blood flow to that vascular bed. The fractional right-to-left shunt was calculated as the difference between pulmonary venous and arterial oxygen saturation divided by the difference between pulmonary venous and mixed-venous oxygen saturation. The pulmonary venous oxygen saturation in each hypoxemic lamb was estimated by reading the oxygen saturation corresponding with its alveolar oxygen tension from the lamb's oxygen dissociation curve (Chapter 3). The alveolar oxygen tension was calculated by using the alveolar gas equation:

$$P_{AO_2} = F_{IO_2} (P_B - P_{H_2O}) - P_{ACO_2} \{F_{IO_2} + (1 - F_{IO_2})/ RQ\}$$

where P_B = 760 torr, P_{H_2O} = 47 torr, P_{ACO_2} was assumed to be equal to the arterial P_{CO_2}, F_{IO_2} = 0.2093, and RQ = 0.8 [151].

Organ blood flows (microsphere method)

Microspheres of 15 μm diameter labeled with either ^{141}Ce, ^{103}Ru, ^{51}Cr or ^{95}Nb (NEN-Trac, Dupont Co., Wilmington, DE, USA) were used. The reference sample

was withdrawn from the ascending aorta at a rate of 6-7 mL/min by using a Harvard pump (Harvard Apparatus, Millis, MA, USA), 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 measured with a Beckman 9000 multi-channel gamma-scintillation counter (Beckmann Instruments Inc., Fullerton, CA, USA). Each organ or organ part was counted separately. Blood flow to organs or organ parts was calculated from the ratio of radioactivity counts in the tissue and the reference sample times withdrawal rate of the reference sample, by using a software package [191]. Blood flow rates were expressed in mL/min and per 100g of tissue. Adequate mixing of microspheres was checked by ascertaining that the calculated blood flow per unit mass to the left and right cerebral hemisphere differed by no more than 10 % [108]. Endocardial to epicardial blood flow ratio of the left and right ventricular free wall was calculated as the ratio of the blood flows per unit mass to the respective endocardial and epicardial layers. Hepatic arterial blood flow was calculated from the microspheres that lodged in the liver. Total hepatic blood flow was calculated as the sum of hepatic arterial blood flow and portal venous blood flow. Portal venous blood flow was calculated as the sum of blood flows to the stomach, the small and the large intestines, the spleen and the pancreas. Renal plasma flow was calculated by multiplying renal blood flow with {100-hematocrit(%)}/100.

In two hypoxemic lambs, that were used in the experiment described in Chapter 4, additional measurements were made to determine whether microspheres reached the lungs either by means of shunting across the foramen ovale during injection of microspheres, or by means of increased bronchial arterial blood flow, or by means of systemic arteriovenous shunting. In these animals additional catheters were inserted, under local anesthesia with lidocaine, the day before the measurements. The catheters were inserted into the left ventricle through the right carotid artery, and into the descending aorta, with the tip located just above the diaphragm, through the femoral artery. After all control measurements were obtained, microspheres each with a different label were simultaneously injected into the left atrium and into the left ventricle. We assumed that microspheres injected into the left ventricle would not shunt across the foramen ovale during injection, but reach the lungs either through the bronchial arterial circulation or through systemic arteriovenous shunting. In addition to the arterial (aortic) reference sample, we obtained a reference sample from the pulmonary artery and calculated the concentration ratio of microspheres in the pulmonary arterial and the aortic reference sample. We assumed that the microspheres in the pulmonary artery reference sample were obtained from shunting across the foramen ovale or

from systemic arteriovenous shunting. Furthermore, microspheres were slowly injected into the descending aorta, to determine the presence of systemic arteriovenous shunts. The number of microspheres that lodged in the lung was expressed as a fraction of the number of microspheres that lodged in organs below the diaphragm.

Whole blood viscosity and vascular hindrance

Fifteen mL of blood were withdrawn and immediately transferred to a dry EDTA containing tube. Whole blood viscosity was measured while shear rates slowly increased up to 200 s^{-1} by using an oscillating capillary viscometer (type OCR-D, A. Paar K.G., Graz, Austria). All measurements were made at 39 °C within 4 h after sampling of the blood. In the time between sampling and measurement, the blood was kept at 39 °C in a water bath. The vascular hindrance, which was used as an estimate of vascular tone, was calculated as resistance divided by whole blood viscosity and expressed in g/mL. Because a large part of the resistance in tissues is located at the arteriolar level and the shear rates are high in this part of the vascular tree [78], we used the whole blood viscosity measured at a shear rate of 100 s^{-1}.

Catecholamines, left ventricular oxygen and substrate uptake

Four mL of blood were obtained from the ascending aorta to measure epinephrine and norepinephrine concentrations. Samples were centrifuged at 4 °C for 10 min and the thrombocyte-poor plasma was transferred to tubes containing glutathion as antioxidant, stored at - 20 °C and measured within 7 days by HPLC with electrochemical detection [205].

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 it was deproteinized with perchloric acid, neutralized with KOH and morpholinopropansulfonic acid solution, centrifuged and immediately stored in ice. Glucose, pyruvate, lactate, β-hydroxybutyrate and acetoacetate concentrations were measured in triplicate with NAD(P)/NAD(P)H-linked enzymatic methods [25]. 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 was immediately transferred to a chilled tube containing dried NaF, centrifuged and the plasma was frozen to -70 °C. Plasma free fatty acid concentration was determined enzymatically by means of a commercial kit (NEFAC, Wako Chemicals GmbH, Neuss, Germany) [53]. Free fatty acid concentration in whole blood was calculated by multiplying plasma concentration with {100 - hematocrit (%)}/100.

The arterio-venous concentration difference across the left ventricle (LVAVD) for oxygen and substrates was calculated as the difference between ascending

aortic and coronary sinus concentrations. Because the blood sampled from the coronary sinus is mainly derived from the left ventricular free wall [56], 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 oxygen extraction ratio (OER) for each substrate was calculated using the formula:

$$OER = (LVAVD_{substrate}/LVAVD_{oxygen}) \times n_{substrate}$$

The factor $n_{substrate}$ is equal to the amount of oxygen required to completely oxidize one mol of substrate: $n_{glucose}=6$, $n_{pyruvate}=2.5$, $n_{lactate}=3$, $n_{acetoacetate}=4$, $n_{\beta\text{-hydroxybutyrate}}=4.5$, and $n_{free\ fatty\ acids}=25$ mol/mol. The rate pressure product (heart rate times systolic arterial blood pressure) and stroke work (approximated as stroke volume times systolic arterial blood pressure) were calculated as indices of left ventricular oxygen demand.

Oxygen affinity and 2,3-DPG concentration

Fifteen mL of heparinized blood were obtained from each lamb that participated in the study described in Chapter 3. The oxygen dissociation curve (ODC) of whole blood was determined in a system in which P_{O_2} and oxygen saturation were measured continuously and independently at a constant temperature of 39 ± 0.02 °C, a pH of 7.40, and a P_{CO_2} of 5.33 kPa, as described by Zwart et al. [248]. Eight mL of heparinized blood were brought into a measuring chamber and continuously stirred. A variable mixture of pure N_2 and pure O_2 each containing 5.6% CO_2 was led through the chamber, while the P_{O_2} of the gas mixture is slowly increased from 0 to >55 kPa. The P_{O_2} is measured by a fast-responding Clark-type electrode and the oxygen saturation by a fiber optic reflection oximeter. The pH of the blood in the chamber was measured continuously and the signal is transmitted to a titration system including an autoburette that contained 1 mol/L NaOH. In this way the protons liberated during the oxygenation of hemoglobin were continuously neutralized and thus the ODC recorded at constant pH. During the recording of the ODC, the P_{O_2} and the oxygen saturation were sampled every 0.2 s and stored in a data acquisition system. Over 2500 data points were sampled for each ODC. The proton Bohr-factor was determined by measuring P_{O_2} at a constant saturation of 50 % and a P_{CO_2} of 5.33 kPa, while increasing the pH from 7.0 - 7.6 in steps of 0.2.

The position of the ODC was expressed as standard P_{50} ($P_{50_{st}}$), which is the P_{O_2} corresponding to an oxygen saturation of 50 % at 39 °C, pH=7.40 and P_{CO_2}=5.33 kPa. The actual P_{50} ($P_{50_{act}}$) for each lamb was calculated from the $P_{50_{st}}$ corrected for actual body temperature, arterial pH, and P_{CO_2}

$$\log P_{50_{act}} = \log P_{50_{st}} + \Delta \log P_{O_2}$$

$$\Delta \log P_{O_2} = \phi H \cdot \Delta pH + \phi C \cdot \Delta \log(P_{CO_2}) + (d \log P_{O_2}/dT)\Delta T$$

ϕH is the proton-Bohr factor and ϕC the carbamate-Bohr factor [131]. For ϕH the proton-Bohr factor of each individual lamb was used, for ϕC a factor of 0.05 [131], and for $(d \log P_{O_2}/dT)$ a factor of 0.024 [249]. The 2,3-DPG concentration was measured by means of a kit (Sigma Chemical Co., St. Louis, MO, USA) according to the enzymatic method of Keitt [122]. The 2,3-DPG concentration was expressed as the molar ratio of 2,3-DPG and Hb_4.

Body fluid compartment volumes

One mL/kg of a mixture of 0.4 mmol/L Evans Blue (Merck, Darmstadt, Germany), 100 mmol/L sodium ferrocyanide (BDH Chemicals, Poole, England) in deuteriumoxide (D_2O) 99.8% (Merck, Darmstadt, Germany) was injected to determine the plasma volume, the extracellular water volume and the total body water volume, respectively. The exact amount of indicator injected was determined by weighing the syringe containing the indicator mixture before and after the injection. After treatment of the samples as described below, light absorbances were measured and converted to concentrations by using calibration lines that were determined before each set of measurements as described by Zweens et al. [250-252].

The Evans Blue concentration was measured in the samples withdrawn at 20, 30, 40, 50 and 60 min. Polyethylene glycol 240 g/L (0.8 mL) was mixed with plasma (0.8 mL) and allowed to stand for 10 min. The mixture was then centrifuged at 7000 g for 10 min and the absorbance of the supernatant measured at 620 nm with a Hitachi 100-40 spectrophotometer (Hitachi Ltd., Tokyo, Japan) against a similarly treated plasma blank. The concentration at time zero (c_0^{EB}) was calculated by extrapolation from the mono-exponential concentration versus time curve. Since Evans Blue is completely mixed with plasma 10 min after injection and subsequently disappears from plasma in a first-order fashion, the error made in c_0^{EB} by assuming mono-exponential elimination between 20 and 60 min after injection is negligible [250]. The plasma volume (V_p) was calculated as:

$$V_p = m_i^{EB}/c_0^{EB}$$

m_i^{EB} denotes the amount of Evans Blue injected.

The sodium ferrocyanide concentration was determined in all samples. A solution (4.5 mL) containing trichloric acetic acid (0.14 mmol/L) and perchloric acid (1.10 mmol/L) was mixed with 0.5 mL plasma and allowed to stand for 10 min. Subsequently, it was centrifuged for 10 min at 7000 g, then 1 mL of a solution

containing $FeSO_4$ (5 g/L) and H_2SO_4 (90 mmol/L) was added to 4 mL of supernatant. After 25 min a blue color had developed that remained stable for at least 15 min. During this period the absorbance of the solution was measured against a reagent blank at 700 nm by means of an Optica CF4 spectrophotometer (Optica, Milan, Italy). The plasma concentrations (c_p^{FC}) were converted to plasma-water concentrations (c_{pw}^{FC}), by correcting for plasma protein concentration:

$$c_{pw}^{FC} = c_p^{FC} x 1000/(1000-0.75 x c_p^{TP})$$

c_p^{TP} is total plasma protein concentration (g/L) and 0.75 the specific volume of protein (mL/g). The time-concentration curve obeyed a tri-exponential model:

$$c_t = Ae^{-k_1 t} + Be^{-k_2 t} + Ce^{-k_3 t}$$

A, B, and C are the coefficients, and k_1, k_2, and k_3 the exponents of each of the mono-exponential parts of the curve, which can be determined by curve stripping. The extracellular water volume (V_{ec}) was then calculated:

$$V_{ec} = m_i^{FC} \{(A/k_1^2 + B/k_2^2 + C/k_3^2)/(A/k_1 + B/k_2 + C/k_3)^2\}$$

The symbols are identical to those in the previous equation and m_i^{FC} denotes the amount of sodium ferrocyanide injected.

To determine the D_2O concentration, about 0.5 mL of red cells were vacuum-sublimated to near dryness and the condensate was trapped in tubes immersed in liquid nitrogen. The absorbance of the condensate was determined against a reference of ordinary window glass at 4023 nm by a Perkin-Elmer 177 infrared-spectrophotometer (Perkin Elmer Corp., Norwalk, CT, USA) [252]. Since the distribution phase of D_2O is short ($\approx$ 40 min) relative to the elimination phase ($t_{1/2} \approx$ 6 days) [252], $c_0^{D_2O}$ can be calculated assuming mono-exponential elimination. In 4 lambs D_2O concentrations were determined in all samples and no observable change in D_2O concentration was found after 40 min. Therefore, in the subsequent experiments we calculated $c_0^{D_2O}$ as the mean of the concentrations at 50 - 120 min. Body water volume (V_{bw}) was calculated as:

$$V_{bw} = m_i^{D_2O}/c_0^{D_2O}$$

$m_i^{D_2O}$ denotes the amount of deuteriumoxide injected.

Plasma osmolality was determined in duplicate by means of an Osmomat 030 (Gonotec GmBH, Berlin, Germany), concentration of total protein by an automatic chemical analyzer ACA III (DuPont Company, Wilmington, DE, USA) and of sodium

by flame photometry (IL343, Instrumentation Laboratory Inc., Lexington, MA, USA). Since ferrocyanide is freely filtered by the glomeruli and neither excreted nor reabsorbed by the tubules, the glomerular filtration rate was calculated from the slope of the mono-exponential tail of the disappearance curve [251].
Blood volume (V_b) was calculated as: $V_b = V_p x 100/(100\text{-hematocrit} \times 0.92)$
the factor 0.92 corrects for the difference between the arterial and the total body hematocrit [179].
The total red cell volume ($V_{red\ cell}$) was calculated as: $V_{red\ cell} = (V_b - V_p)$.
The total amount of hemoglobin as: {V_b (l) x hemoglobin concentration (g/L)}.
The interstitial water volume (V_{int}) as: $V_{int} = (V_{ec} - V_p)$.
The intracellular water volume (V_{ic}) as: $V_{ic} = (V_{bw} - V_{ec})$.
The mass of the solids as: (body mass - total body water mass), assuming a density of water of 1.00 kg/L.
To determine the effects of hypoxemia on intracellular water volume, not related to alterations in red cell mass, we calculated the intracellular volume that was extravascular: $V_{ic}^{extravascular} = V_{ic} - (V_{red\ cell} - V_{hemoglobin})$.
$V_{hemoglobin}$ was calculated assuming a similar specific volume for hemoglobin as for plasma protein (0.75 mL/g).

Statistical analysis

The cardiovascular and hematological variables and oxygen uptake that were repeatedly measured in each lamb during an experiment were averaged, so that one value for each variable for each lamb was obtained. Subsequently, mean and SD were calculated for control and hypoxemic lambs. Control and hypoxemic lambs were compared by using an unpaired Student t-test. In two experiments (Chapter 3 and 5) control lambs were studied that either underwent surgery or that had neck vessels catheterized. Control and hypoxemic lambs were compared by one-way ANOVA in these experiments. If the F-value was higher than the critical value, the Student-Newman-Keuls test or multiple contrasts were used to test for the effects of surgery and hypoxemia. The data of all control lambs were pooled if no differences between the subgroups could be demonstrated, and an unpaired Student t-test was performed between control and hypoxemic lambs. In a number of experiments linear regression or multiple linear regression was used if appropriate. If multiple linear regression was used, a dummy variable was introduced to represent control and hypoxemic lambs; in this way the effects of hypoxemia could be tested in multiple linear regression [246]. In all analyses $p<0.05$ was chosen as the level of significance.

3

Systemic oxygen supply, oxygen uptake, and oxygen affinity in chronically hypoxemic lambs

Congenital cyanotic heart disease is an important cause of chronic hypoxemia in childhood. Several factors contribute to maintain the balance between oxygen supply and oxygen demand, but their relative importance is still not clear. Systemic oxygen supply is maintained by the increase in hemoglobin concentration, whereas systemic blood flow is generally not increased [26, 82]. However, the extent to which the increased hemoglobin concentration compensates for the decreased arterial oxygen saturation varies [26]. When systemic oxygen supply is decreased, oxygen demand may be met by an increase in oxygen extraction, which may be facilitated by a decrease in the oxygen affinity of hemoglobin. The oxygen affinity in chronically hypoxemic subjects has been reported to be either decreased or normal and its impact on the adjustment to chronic hypoxemia is unclear [26, 82, 167, 231]. The variable results reported for cyanotic subjects can in part be explained by heterogeneity with respect to age, the severity of hypoxemia, and by the presence or absence of fetal hemoglobin.

Developmental changes in cardiovascular and hematological variables, and the response to acute stress have been widely studied in lambs [63, 137, 138, 161, 201]. During the first weeks of life these variables are subject to rapid change and the ability to tolerate a decrease in oxygen supply is relatively low [63, 138]. In lambs of 5-6 weeks and older, however, oxygen supply and oxygen uptake are stable and oxygen affinity is similar to that in adult sheep [138]. Teitel et al. described a model to study chronic hypoxemia in lambs with experimental cyanotic heart disease [213]. These lambs were studied for two weeks after the induction of hypoxemia until they were 4 weeks old. The major sequelae of chronic hypoxemia were an increased hemoglobin concentration, a decreased growth rate, and an increased mortality, whereas no difference in the calculated oxygen affinity was found. However, the stress of surgery, the subsequent induction of chronic hypoxemia, and the repeated measurements in a period of limited reserve to cope with additional stress, may have limited the ability to adjust to chronic hypoxemia in these lambs. Moreover, measurements were made at a time when the fraction of fetal hemoglobin still was high, which may have affected the oxygen affinity.

It was our purpose, therefore, to study the cardiovascular and hematological adjustments to chronic hypoxemia in lambs after these variables had become stable, and at a time when the fraction of fetal hemoglobin would be low. We determined the role of a decreased oxygen affinity as an adjustment to chronic hypoxemia by measuring the oxygen dissociation curve in each lamb. We expected that an increased hemoglobin concentration would be the major adjustment to chronic hypoxemia, but that a decreased oxygen affinity might

contribute to the adjustment to chronic hypoxemia as well. In addition, we evaluated the effects of surgery on cardiovascular and hematological variables and on growth, by including a group of control lambs that only underwent neck vessel catheterization.

Materials and methods

Thirteen control lambs and 10 chronically hypoxemic lambs were studied. Six control lambs and all hypoxemic lambs underwent a left thoracotomy before the 10th day of life. The other 7 control lambs stayed at a farm until a few days before the measurements. In these lambs neck vessel were catheterized under local anesthesia, at least 24 h before the measurements. Statistical analysis was performed by one-way ANOVA to analyze the results of the two groups of control lambs (with and without surgery) and the hypoxemic lambs. In addition, the pooled data of all control lambs were compared with those of hypoxemic lambs by unpaired Student's t-test. For further details the reader is referred to Chapter 2.

Results

Age, body mass, cardiovascular and hematological variables for hypoxemic and the two groups of control lambs, are shown in Table 3.1. Because no differences

Table 3.1 General, hematological and cardiovascular variables for control lambs that underwent surgery, for lambs that only had their neck vessels catheterized (no surgery), and for chronically hypoxemic lambs.

	Control surgery n=6	Control no surgery n=7	Chronic hypoxemia n=10
Age (d)	36±4	36±2	35±4
Weight gain (g/day)	196±44	220±39	144±75
Oxygen saturation (%)			
arterial	93±2	93±2	66±8*
mixed-venous	59±3	58±1	41±10*
Hemoglobin (g/L)	112±11	105±20	144±13*
Hematocrit (%)	34±1	32±5	44±4*
Oxygen uptake (mL/min·kg)	8±2	8±2	9±2
Mean pressures, mmHg			
aortic	79±11	82±10	76±12
pulmonary	16±3	11±2	14±4
Heart rate (bpm)	130±23	137±20	176±23*
Systemic blood flow (mL/min·kg)	158±32	171±38	173±72

All data are mean ± SD. * Hypoxemia significantly different from control by ANOVA and the Newman-Keuls test, $p<0.05$.

were found between the two groups of control lambs, the data of all control lambs were pooled and compared with those of the hypoxemic lambs.

Systemic oxygen supply and its determinants are shown in Figure 3.1. The decreased arterial oxygen saturation was compensated for by an increase of the hemoglobin concentration in hypoxemic lambs, so that the arterial oxygen concentration was similar to that in control lambs. Likewise, systemic blood flow, systemic oxygen supply, oxygen uptake, and oxygen extraction were similar in the two groups of lambs, but mixed-venous oxygen saturation (Table 3.1) and mixed-venous P_{O_2} (Table 3.3) were decreased in hypoxemic lambs. Heart rate was increased and left ventricular stroke volume was decreased in hypoxemic lambs,

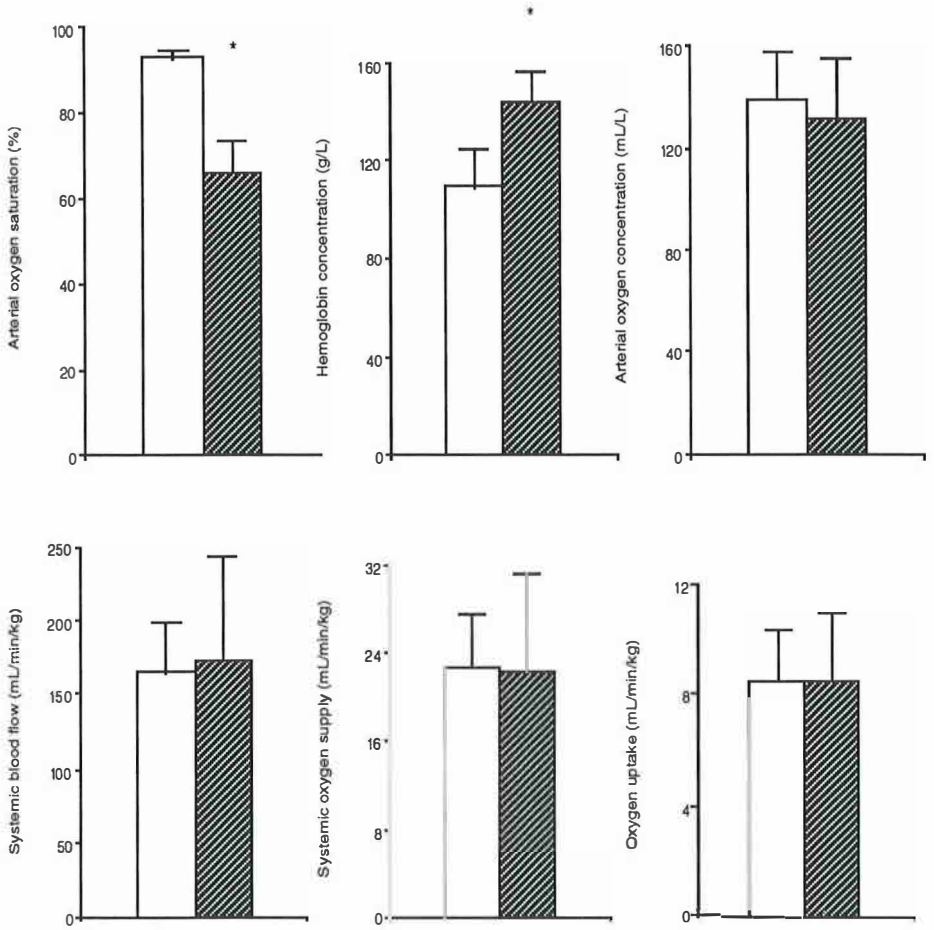

Figure 3.1 Determinants of systemic oxygen supply and oxygen uptake in control (open bars) and hypoxemic lambs (hatched bars). * $p<0.05$.

Table 3.2 Hemodynamic variables.

	Control n=13	Hypoxemia n=10
Mean blood pressures (mmHg)		
arterial	79±9	77±12
pulmonary	13±3	15±4
right atrial	2±1	6±4
left atrial	7±6	6±4
Heart rate (bpm)	136±21	176±23*
Systemic blood flow (mL/min·kg)	166±33	173±72
Left ventricular stroke volume (mL/kg)	1.2±0.2	1.0±0.3*
Systemic vascular resistance (mmHg·min·kg/L)	489±125	501±264

* Hypoxemia significantly different from control by unpaired Student's t-test, $p<0.05$.

blood pressures and systemic vascular resistance were similar in hypoxemic and control lambs (Table 3.2).

The $P50_{st}$ ranged from 4.0 to 5.8 kPa in the lambs and was not significantly different in hypoxemic as compared with control lambs (5.05±0.45 *versus* 5.15±0.43 kPa). Similarly, the $P50_{act}$ was not significantly different in hypoxemic as compared with control lambs (5.38±0.63 *versus* 5.68±0.80 kPa). The 2,3-DPG concentration was low in both groups of lambs (0.13±0.05 *versus* 0.10±0.09 mol/mol Hb_4) and the proton Bohr-factor was similar in hypoxemic and control lambs (-0.31±0.03 *versus* -0.32±0.05). The P50 was not related to 2,3-DPG concentration and lambs with a high P50 did not have a lower arterial or mixed-venous oxygen concentration than lambs with a low P50.

Hypoxemic lambs had a decreased PCO_2 and an increased base deficit (Table 3.3). The calculated alveolar oxygen tension in control lambs was not significantly different from the arterial oxygen tension (paired t-test). In hypoxemic lambs the calculated alveolar oxygen tension was higher than in control lambs

Table 3.3 Blood gases and alveolar oxygen tension.

	Control	Hypoxemia
Arterial		
pH	7.41±0.03	7.39±0.02
PCO_2 (kPa)	4.9±0.3	4.0±0.7*
PO_2 (kPa)	13.8±1.2	8.2±1.0*
base excess (mmol/L)	-1±2	-6±3*
Mixed-venous		
PO_2 (kPa)	7.2±0.5	5.9±1.0*
Alveolar oxygen tension (kPa)	14.4±0.4	15.6±0.8*

* Hypoxemia significantly different from control by unpaired Student's t-test, $p<0.05$.

(Table 3.3), but the estimated pulmonary venous oxygen saturation in hypoxemic lambs (93±2 %) was similar to the arterial oxygen saturation in control lambs. The right-to-left shunt was estimated 49±10 % of systemic blood flow.

Body mass was lower in hypoxemic than in control lambs (9.9±2.5 *versus* 11.5±1.9 kg), but the difference was not statistically significant. However, the mean increment in body mass from the day of surgery to the day of study was 30 % lower in hypoxemic lambs (23±10 *versus* 36±12 g/kg·day, $p<0.01$; Fig. 3.2).

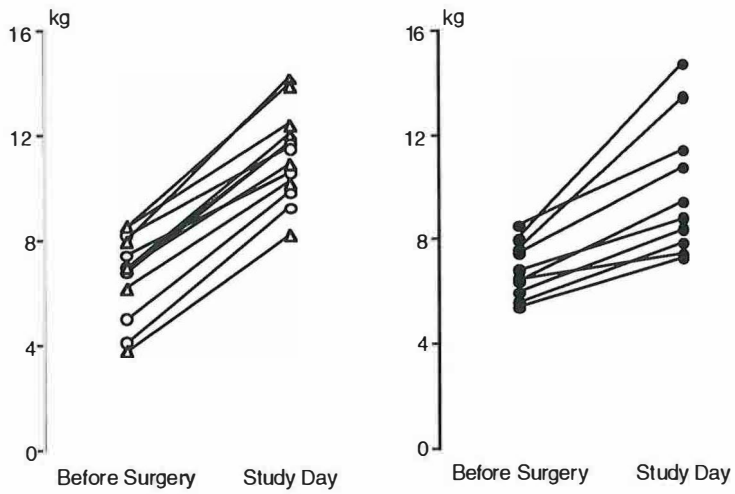

Figure 3.2 Body mass before surgery, around the 10th day of life, and on the day of study in control lambs that underwent surgery (open dots), control lambs that underwent neck vessel catheterization (open triangles) and in hypoxemic lambs (solid dots). The variability in body mass in both groups of lambs persists throughout the study period, but the increase in body mass is higher in control than in hypoxemic lambs.

In preliminary experiments the hemoglobin concentration measured by the Radiometer OSM2 tended to be higher in the mixed-venous and the coronary-sinus blood samples than in the arterial blood samples. To distinguish between a methodological artifact and hemoconcentration across the systemic and coronary circulation, we compared hemoglobin concentration measured by the cyanomethemoglobin method with that measured by the OSM2. Both in the control and hypoxemic lambs the hemoglobin concentration measured by the cyanomethemoglobin method was constant, whereas it increased with decreasing oxygen saturation when measured by the OSM2 (Table 3.4). With the cyanomethemoglobin method the hemoglobin concentration was independent of the oxygen saturation both in the hypoxemic and control lambs by multiple linear regression (partial $F=0.9$, $p=0.3$). In contrast with the OSM2, the hemoglobin

Table 3.4 Comparison of hemoglobin concentration measured by the cyanomethemoglobin method and by the Radiometer-OSM2.

	Oxygen saturation (%)	Hemoglobin concentration (g/L) HbHiCN	OSM2
Control lambs			
arterial (n=22)	92±3	115±10	109±10
mixed-venous (n=20)	59±4	113±9	113±9
Hypoxemic lambs			
arterial (n=32)	65±11	139±9	143±12
mixed-venous (n=30)	38±12	139±10	147±12
coronary sinus (n=28)	16±4	138±10	151±13

n pertains to the number of samples that was obtained from each site in eleven control and in eight hypoxemic lambs. HbHiCN = cyanomethemoglobin method.

concentration increased when the oxygen saturation decreased both in the hypoxemic and control lambs (partial F=12.8, p<0.001). By either method the hemoglobin concentration was higher in hypoxemic than in control lambs (p<0.001). Thus, the hemoglobin concentration that is measured by the OSM2 depends in part on the oxygen saturation of the sample that is analyzed. The extent of the methodological error made by the OSM2 is demonstrated in Fig. 3.3.

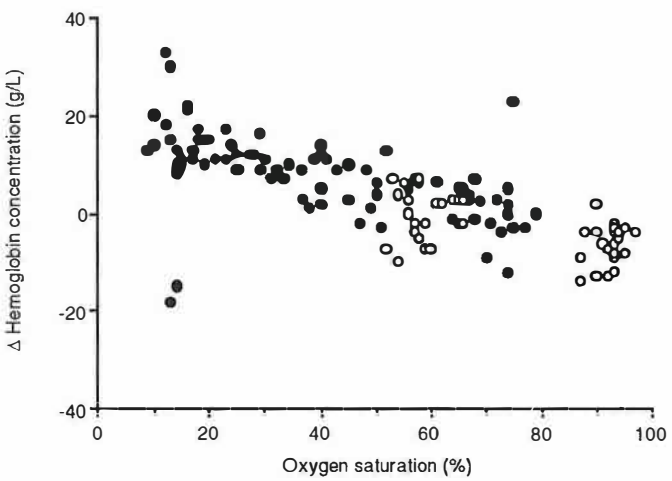

Figure 3.3 Difference between the hemoglobin concentration measured with the Radiometer OSM2 and with the cyanomethemoglobin method (Δ hemoglobin concentration) in control (open dots) and hypoxemic lambs (solid dots) as a function of the oxygen saturation. The slope of the regression is highly significant (-0.25±0.02 (SE), p<0.001).

Discussion

In this study we demonstrated that the increased hemoglobin concentration is the major adjustment to chronic hypoxemia in lambs and that oxygen affinity of hemoglobin is not decreased. Moreover, growth rate is decreased in hypoxemic lambs and this can be attributed to the effects of hypoxemia.

Before discussing the results, three methodological considerations deserve comment. First, hypoxemia was induced in a period of rapid growth and hematological and cardiovascular changes [138]. Surgery in the first ten days of life and the stay in the laboratory may have interfered with a normal development of the lambs. To assess these effects we studied two control groups. The first group underwent surgery before the 10th day of life and stayed in the laboratory for 3-4 weeks, like the hypoxemic lambs. The second group was not subjected to surgery and remained at a farm until a few days before the study. Because there were no differences between these two groups it is clear that surgery in the first ten days of life and the stay in the laboratory did not interfere with a normal development of the lambs. Thus, the differences between hypoxemic and control lambs can be attributed to the effects of hypoxemia.

Second, we wanted to determine whether chronic hypoxemia induced a decrease in oxygen affinity. In newborn lambs the oxygen affinity is high, but it decreases gradually as high-affinity fetal hemoglobin is replaced by low-affinity adult hemoglobin. In 5- to 6-week old lambs the fraction of fetal hemoglobin is low and the oxygen affinity is at the adult level [138]. The effect of an increased erythropoiesis after the induction of hypoxemia on the decrease of the fraction of fetal hemoglobin, depends on the production of fetal hemoglobin relative to adult hemoglobin [11]. In humans this switch occurs gradually over weeks [172], but at which rate it occurs in sheep is not exactly known. Hence, the rate at which the fraction of fetal hemoglobin and thus oxygen affinity decreases in chronically hypoxemic lambs is not exactly known. However, the P_{50} in our hypoxemic lambs was comparable to that in control lambs, while the 2,3-DPG levels were low in both groups of lambs, indicating that the fraction of fetal hemoglobin must have been low in our hypoxemic lambs.

Third, we assessed the accuracy of the Radiometer-OSM2 for the measurement of the hemoglobin concentration. Our results demonstrate that the OSM2 is not suitable to measure the hemoglobin concentration, especially not when studying (chronic) hypoxemia, because the measurement depends on the oxygen saturation. The wavelength at which the hemoglobin concentration is measured is obviously not quite isosbestic, light absorption by deoxy-hemoglobin being greater than by oxy-hemoglobin [227].

Although systemic oxygen supply and oxygen uptake were well maintained in chronically hypoxemic lambs, these adjustments may be established at the

expense of the reserve to cope with additional perturbations in the oxygen transport system. An increase of the systemic blood flow and the oxygen extraction are both important factors for maintaining an adequate tissue oxygen supply during an acute decrease of the arterial oxygen concentration or an increase of the oxygen demand [68, 106, 201]. In hypoxemic lambs the ability to maintain oxygen supply to the tissues may under these circumstances be impaired for several reasons. First, heart rate is increased in hypoxemic as compared with control lambs, but the maximal heart rate will not be higher [28]. Consequently, the reserve to increase heart rate will be lower in hypoxemic than in control lambs. Second, left ventricular stroke volume is lower in hypoxemic than in control lambs. This is not explained by a decreased left atrial pressure in hypoxemic lambs. A decrease of the myocardial contractility may explain the decreased left ventricular stroke volume during hypoxemia [2], but no clear alterations in cardiac function have been demonstrated during extreme hypoxemia [177]. Right ventricular dilatation [132] or right ventricular outflow tract obstruction [199] may impair left ventricular filling and induce a decrease of the left ventricular stroke volume. The right ventricular hypertrophy in our hypoxemic lambs probably contributes to the decreased left ventricular stroke volume, by interfering with left ventricular filling. Because hypoxemic lambs cannot increase their heart rate and their left ventricular stroke volume as much as control lambs, it is conceivable that they have a limited ability to increase their systemic blood flow.

To increase oxygen extraction, mixed-venous oxygen saturation must decrease. Hypoxemic lambs at rest maintain their arterio-mixed-venous oxygen concentration difference at the expense of a lower mixed-venous oxygen saturation and P_{O_2}. A low oxygen tension may limit the diffusion of oxygen into the tissues. For example, when 1- to 6-week-old lambs are subjected to acute hypoxemia, the oxygen uptake decreases and the mixed-venous P_{O_2} falls to a similar level in all lambs, despite differences in oxygen affinity and oxygen extraction [201]. Similarly, when fetal hemoglobin is replaced by adult hemoglobin during acute hypoxemia in newborn lambs, the mixed-venous P_{O_2} is unchanged, but the oxygen uptake increases to values found before the induction hypoxemia [137]. These results suggest that the mixed-venous P_{O_2} cannot decrease any further during acute hypoxemia to maintain oxygen uptake. Because the arterial oxygen concentration in our hypoxemic lambs is at the same level as in control lambs, the mixed-venous P_{O_2} will be lower in hypoxemic lambs if both groups unload the same amount of oxygen (Fig. 3.4). If tissue hypoxia develops at the same levels of mixed-venous P_{O_2} (the critical mixed-venous P_{O_2}) in hypoxemic and control lambs, the mixed-venous P_{O_2} in hypoxemic lambs in our study is closer to this critical level. Conversely, at the critical mixed-venous P_{O_2} hypoxemic lambs will have unloaded less oxygen than control lambs. However, this critical mixed-venous P_{O_2}

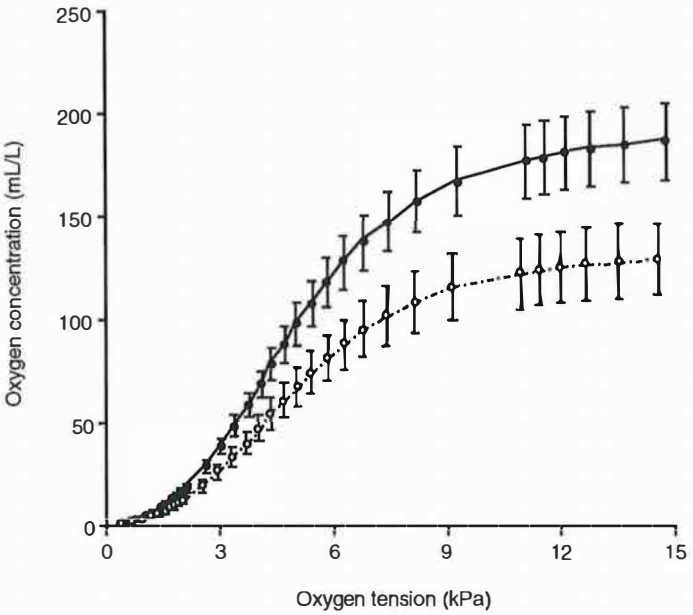

Figure 3.4 Oxygen concentration of blood in hypoxemic and control lambs plotted as a function of PO_2 (at 39 °C, pH=7.40, PCO_2=5.33 kPa). At each PO_2 the oxygen concentration is higher in the blood of hypoxemic lambs, because of the increased oxygen capacity. Due to the increased oxygen capacity in the hypoxemic lambs more oxygen can be unloaded for the same fall in oxygen tension as compared with control lambs.

presumably is around 1-2 kPa [37, 137, 201], so that the extraction reserve of chronically hypoxemic lambs is similar to that in control lambs (Fig. 8.1).

Oxygen affinity is primarily determined by the intrinsic properties of hemoglobin, but modified by temperature, PCO_2, pH, and 2,3-DPG concentration. In most mammalian species deoxy-hemoglobin is stabilized by an allosteric effect of 2,3-DPG, but in sheep this effect is lacking [187]. However, a high 2,3-DPG concentration may decrease the oxygen affinity by a Bohr-effect. In newborn lambs the intra-erythrocytic 2,3-DPG concentration is high and consequently the intra-erythrocytic pH is low [16, 18, 138]. Thus, lambs do modulate their oxygen affinity by increasing the 2,3-DPG concentration, although the mechanism is different from that in most other mammalian species.

In sheep, three types of adult hemoglobin can be distinguished (HbAA, HbAB, and HbBB), each with an intrinsically different P50 of 4.38 kPa, 4.98 kPa, and 5.70 kPa, respectively [18, 114, 138]. The $P50_{st}$ in the lambs in our study ranged from 4.0 to 5.8 kPa and was not related to 2,3-DPG concentration. Most likely the differences in P50 in our study are related to the intrinsic differences in the three types of adult

hemoglobin. Despite the wide range of P_{50}, the lambs with a high P_{50} (low oxygen affinity) had no clear advantage over lambs with a low P_{50} in their adaptation to hypoxemia. This suggests that, at least in lambs, oxygen affinity has no major impact on the ability to adjust to chronic hypoxemia. The P_{50} of the blood in lambs is considerably higher than in humans and dogs [26, 139, 231, 248], but lambs do not seem to tolerate chronic hypoxemia any better than humans and dogs. The increase in P_{50} in chronically hypoxemic humans and dogs is generally 0.5 kPa or less [26, 139, 231] and it is not a consistent finding in hypoxemic humans [26, 82, 167]. Thus, a decrease in oxygen affinity or a low oxygen affinity per se does not seem to be a major factor in the adaptation to chronic hypoxemia.

In young lambs oxygen is required for metabolism and growth. Our results suggest that oxygen requirements for resting metabolism are met in hypoxemic lambs, but that oxygen availability for growth may be decreased. Teitel et al. found an oxygen extraction of around 50 % in chronically hypoxemic lambs in the first two weeks after induction of hypoxemia [213], against 39 % in our hypoxemic lambs. This indicates that oxygen extraction can be increased if oxygen requirements are higher. Oxygen requirements for resting metabolism may be increased in hypoxemic lambs because of increased cardiorespiratory work. Heart rate is increased and right ventricular mass is higher (Chapter 6), which will increase myocardial oxygen demand. Hypoxemic lambs had a lower arterial P_{CO_2} and we observed an increased respiratory rate, indicating increased ventilation. Thus, even though oxygen uptake at rest in hypoxemic and control lambs is at similar levels, less oxygen may be available for growth. Teitel et al. reported a decreased growth rate for hypoxemic lambs [213], similar to our results. Oxygen requirements for growth have been estimated to range from 30 % of oxygen uptake at rest in 1-week-old lambs to 10 % in 6-week-old lambs [201] and it has been suggested that in acute experimental conditions, the oxygen uptake related to growth can be shut down [63, 201]. Another factor may contribute to a decreased oxygen availability for growth: all our measurements were performed while the lambs were at rest, but we observed that hypoxemic lambs could not feed with their mothers as long as control lambs and soon became tachypneic. Since a cardiac right-to-left shunt can increase during exercise [203], hypoxemic lambs may experience periods of reduced oxygen supply during daily activity like feeding. Although we cannot exclude a decreased energy intake as a cause of the decreased weight gain in our lambs, a normal energy intake has been demonstrated in chronically hypoxemic lambs that were bottle-fed [27]. In addition, a decreased oxygen availability for growth may be a factor in growth reduction in chronic hypoxemia.

In summary, we have shown that chronically hypoxemic lambs at rest maintain their systemic oxygen supply and oxygen uptake at normal levels. The

most important factor in the adjustment to chronic hypoxemia is the increased hemoglobin concentration, which completely compensates for the decreased arterial oxygen saturation. However, the adaptation to chronic hypoxemia is in part established at the expense of the reserve of the oxygen transport system. The normal oxygen affinity indicates that a decrease in oxygen affinity is not an important factor in the adaptation to chronic hypoxemia. Although the resting oxygen demand is apparently met, the adjustments are not sufficient to maintain a normal growth rate.

4

Effect of increased whole blood viscosity on regional blood flows in chronically hypoxemic lambs

An increase in hemoglobin concentration is an important adjustment to chronic hypoxemia and compensates for the decrease in arterial oxygen saturation [8, 49, 213]. The effect of the consequent increase in whole blood viscosity on blood flow and oxygen supply to the tissues depends on the balance between the increase in resistance (due to the increased viscosity), the perfusion pressure and the vascular tone. In acute experimental polycythemia in dogs, oxygen supply to vital organs was better maintained than to non-vital organs, because the vascular tone in vital organs decreased to compensate for the disproportionate increase in whole blood viscosity [65]. In chronically hypoxemic lambs, blood flow and oxygen supply to vital organs was similar to that in normoxemic lambs, but to non-vital organs it was decreased [31]. Because this redistribution of blood flow was in part similar to that encountered during acute hypoxemia in lambs [201], it was suggested that it was related to a (centrally mediated) increase in vascular tone [31]. However, the increase in whole blood viscosity was not accounted for.

We hypothesized that the redistribution of blood flow in chronic hypoxemia is determined by differences in vascular tone, in response to the effects of the increased whole blood viscosity on blood flow and oxygen supply. The increased whole blood viscosity itself will affect blood flow to each organ to a similar extent, but blood flow distribution will be determined by alterations in local vascular tone, mediated by local metabolic factors [88]. Therefore, we suspect that in chronic hypoxemia blood flow and oxygen supply to organs with a large oxygen extraction reserve will decrease, as a consequence of the increased whole blood viscosity, whereas blood flow and oxygen supply to organs with a limited oxygen extraction reserve are maintained by adjustments in vascular tone. To test our hypothesis, we studied blood flow distribution and whole blood viscosity, in chronically hypoxemic and in control lambs and calculated vascular hindrance (resistance/whole blood viscosity) for each organ as previously described [42, 65].

Materials and methods

Eight control lambs and 10 chronically hypoxemic lambs were studied. All lambs underwent surgery. In two hypoxemic lambs, additional measurements were made, to determine by which route(s) microspheres reached the lungs. Statistical analysis was performed by unpaired Student's t-test and by multiple linear regression. For further details the reader is referred to Chapter 2.

Results

Age, body mass, systemic cardiovascular and hematologic variables, and whole blood viscosity are summarized Table 4.1. Blood flow to the heart and brain was

Table 4.1 Age, weight, hematological, and cardiovascular variables, and pH, and blood gases.

	Control n=8	Hypoxemia n=10
Age (d)	43±7	39±5
Body mass (kg)	13.2±2.8	10.0±2.4*
Hemoglobin concentration (g/L)	109±9	145±10*
Hematocrit (%)	29±3	45±3*
Whole blood viscosity (mPa·s)		
shear rate 100 s^{-1}	3.6±0.6	4.4±0.6*
shear rate 50 s^{-1}	3.8±0.8	4.8±0.8*
shear rate 10 s^{-1}	4.3±1.2	5.6±1.3*
Arterial oxygen saturation (%)	91±3	68±10*
Mixed venous oxygen saturation (%)	55±4	39±14*
Arterial oxygen concentration (mL/L)	135±12	134±22
Oxygen uptake (mL/min·kg)	7.1±1.0	8.4±1.3
Mean blood pressure		
arterial (kPa)	11.3±2.4	10.7±1.1
right atrial (kPa)	0.7±0.5	0.9±0.7
Heart rate (beat/min)	120±22	151±25*
Systemic blood flow (mL/min·kg)	127±33	180±89
Systemic oxygen supply (mL/min·kg)	17±4	24±14
Arterial pH and blood gas analysis		
pH	7.42±0.04	7.38±0.06
PCO_2 (kPa)	5.5±0.6	4.4±0.8*
PO_2 (kPa)	13.4±1.9	7.5±2.3*
bicarbonate (mmol/L)	25.2±2.7	18.7±3.8*

* Hypoxemia significantly different from control by unpaired Student's t-test, $p<0.05$.

similar in hypoxemic and control lambs, whereas blood flow to the kidneys, the gastrointestinal tract, the spleen, and the thyroids was lower in hypoxemic than in control lambs (Fig. 4.1). Blood flow to the small intestine was significantly decreased in hypoxemic lambs (72±22 *versus* 136±54 mL/min·100g, $p<0.05$).
Hepatic blood flow was decreased, due to the lower portal venous blood flow in hypoxemic lambs (377±209 *versus* 741±242 mL/min, $p<0.01$); the contribution of hepatic arterial blood flow was only small in both groups of lambs (24±19 *versus* 43±47 mL/min). The distribution of blood flow within the renal cortex was unaltered in hypoxemic lambs and their renal plasma flow was 50 % lower than in control lambs (176±107 *versus* 344±105 mL/min·100g, $p<0.01$). Because the arterial oxygen concentration was similar in hypoxemic and control lambs the pattern of oxygen supply to organs was similar to the pattern of blood flow distribution.

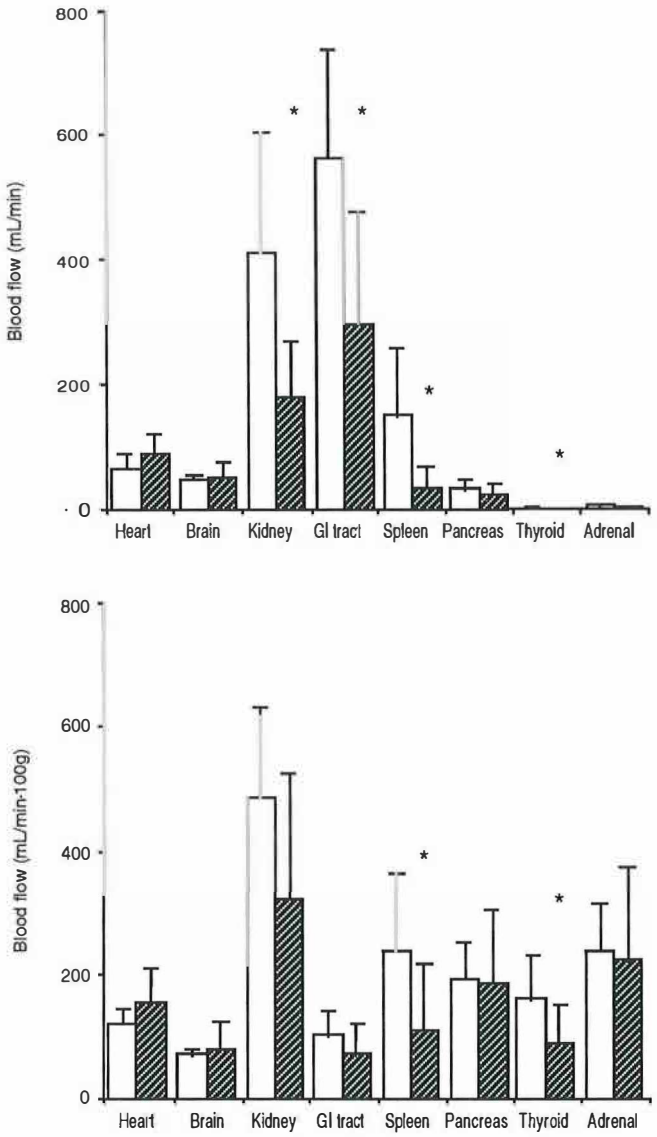

Figure 4.1 Blood flow to organs in mL/min (upper panel) and in mL/min·100g (lower panel) in control (open bars) and hypoxemic lambs (hatched bars). GI tract=gastrointestinal tract. * $p<0.05$.

Resistance in the heart and brain in hypoxemic lambs was similar to that in control lambs, whereas in the kidney, the gastrointestinal tract and the spleen it was significantly increased (Fig. 4.2). Vascular hindrance in the heart was decreased in hypoxemic lambs (Fig. 4.2); the difference was statistically significant for the septum

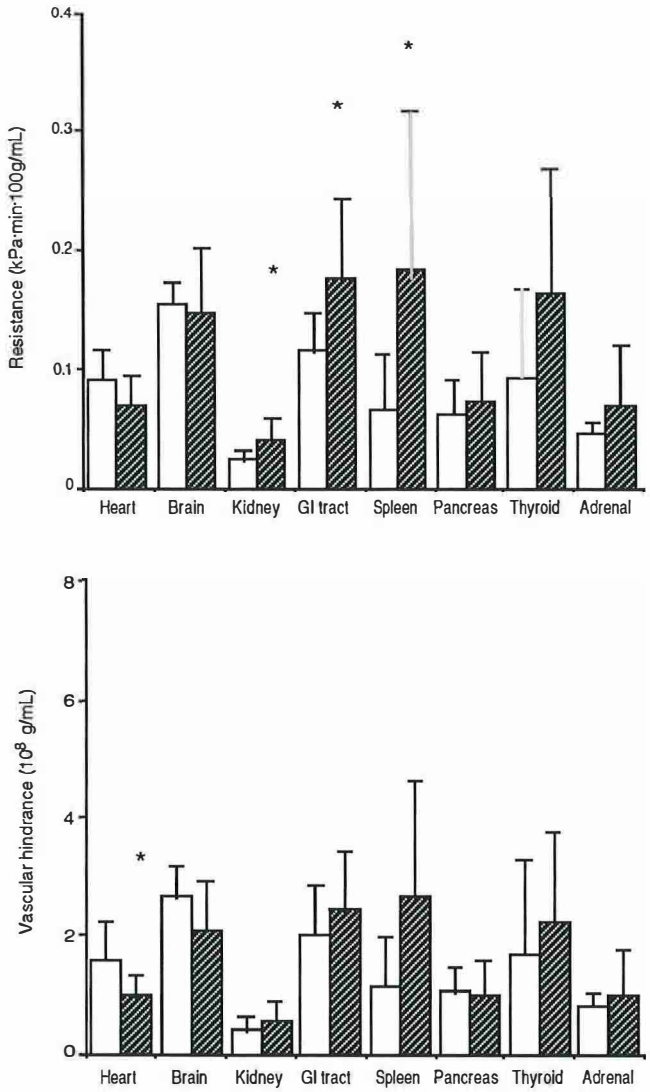

Figure 4.2 Flow resistance (upper panel) and vascular hindrance (lower panel) in control (open bars) and hypoxemic lambs (hatched bars). GI tract=gastrointestinal tract. * $p<0.05$.

and the right ventricular free wall, whereas for the left ventricular free wall it just failed to reach statistical significance ($p=0.06$). Vascular hindrance in the other organs in hypoxemic lambs was not significantly different from that in control lambs (Fig. 4.2).

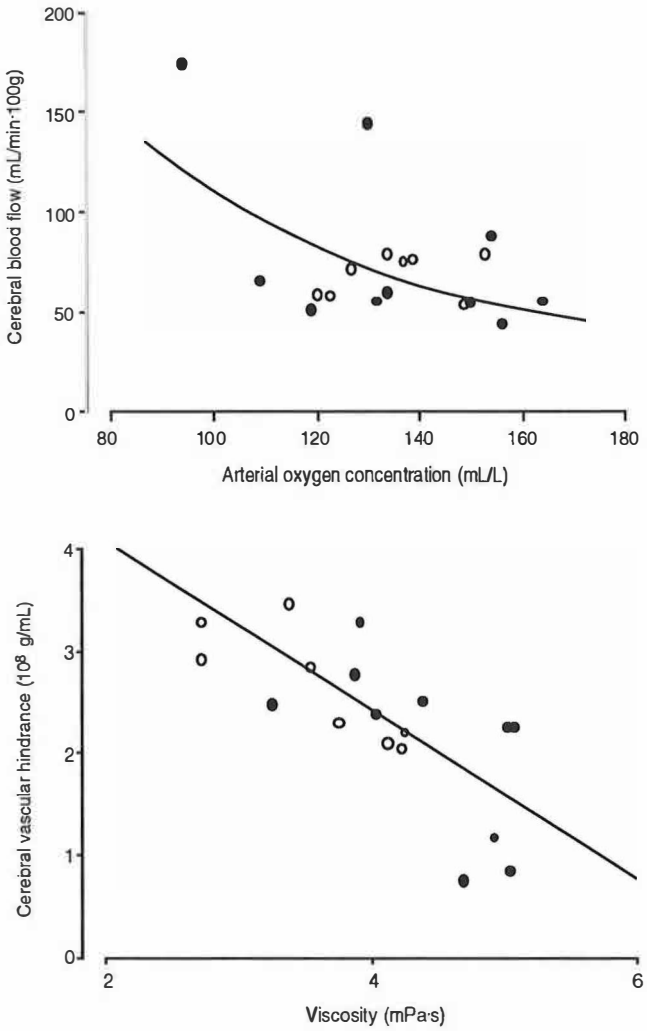

Figure 4.3 Cerebral blood flow as a function of arterial oxygen concentration (upper panel) and cerebral vascular hindrance as a function of whole blood viscosity at shear rate 100 s^{-1} (lower panel) in control lambs (open dots) and hypoxemic lambs (solid dots).

Cerebral blood flow (CBF) increased when the arterial oxygen concentration (Ca_{O_2}) decreased (CBF = 15849±6276(SE)/Ca_{O_2} - 45, $p<0.05$, $r=0.53$; Fig. 4.3), while cerebral oxygen supply was independent of the arterial oxygen concentration. Cerebral vascular hindrance decreased with increasing whole blood viscosity and arterial P_{CO_2} by multiple linear regression ($p<0.001$, $r=0.78$). The relation between whole blood viscosity and cerebral vascular hindrance (Fig. 4.3)

was highly significant (slope of the partial regression: -0.98±0.20, $p<0.001$), whereas for the arterial P_{CO_2} and cerebral vascular hindrance it just did not reach statistical significance (slope: -0.33±0.18, $p=0.08$).

The number of microspheres that lodged in the lungs of hypoxemic lambs was about tenfold higher than in control lambs, but quite variable. In two hypoxemic lambs additional measurements were made to determine by which route(s) microspheres reached the lungs. In the first lamb, the concentration ratio of microspheres in the reference sample in the pulmonary artery and the aorta (after injection of microspheres into the left atrium) was 0.41 and the fractional number of microspheres in the lungs expressed as microspheres in organs below the diaphragm was 0.35. In the second lamb this was 0.02 and 0.06, respectively. This suggests either shunting of microspheres across the foramen ovale during injection or through arteriovenous shunts in the systemic vascular bed. Blood flow to the lungs calculated from the simultaneous injection of differently labeled microspheres into the left atrium and into the left ventricle was practically equal in each lamb: 1452 and 1485 mL/min in the first lamb and 342 and 308 mL/min in the second lamb, respectively. These results suggest that either systemic arteriovenous shunting or increased arterial bronchial blood flow may contribute considerably to the number of microspheres that lodge in the lungs.

Discussion

In this study we demonstrated that, in lambs after 4 weeks of hypoxemia, blood flow to the heart and brain is maintained, whereas blood flow to splanchnic organs, kidneys and thyroid is decreased. The blood flow distribution in the hypoxemic lambs in our study is similar to that reported by Bernstein et al. in lambs after two weeks of hypoxemia [31]. In contrast to the suggestion made by these investigators, our results demonstrate that the redistribution of blood flow in hypoxemic lambs is related to a decrease in vascular tone in vital organs and not to (centrally mediated) vasoconstriction in non-vital organs.

To determine the role of local vascular responses in the redistribution of systemic blood flow in chronically hypoxemic lambs, we calculated the vascular hindrance as a measure of local vascular tone. This method has previously been used to determine the effects of local vascular responses during acute alterations of whole blood viscosity [42, 65]. We expressed the vascular hindrance in g/mL, which demonstrates the direct relation with the vascular geometry. If the decreased blood flow to organs in the hypoxemic lambs would have been related to vasoconstriction, vascular hindrance would have been increased. However, vascular hindrance was similar in most organs in hypoxemic and control lambs, indicating that vascular tone was unaltered and that the decreased blood flow to these organs could be attributed to the increased whole blood viscosity.

The number of microspheres that lodged in the lungs of hypoxemic lambs was considerably higher than in control lambs. In addition, the nutritional blood flow to organs, as estimated by the microsphere method, was decreased in hypoxemic lambs, while their systemic blood flow was similar to that in control lambs. Moreover, blood flow to the carcass and the skin, which comprises about 50 % of systemic blood flow under normal conditions [201], was lower in hypoxemic lambs [31]. To account for the increased number of microspheres in the lungs and the apparent discrepancy between systemic and organ blood flows in hypoxemic lambs, three pathways that microspheres can travel must be considered. First, microspheres may cross the foramen ovale during injection into the left atrium. The magnitude of this shunt will in part depend on the position of the left atrial catheter relative to the foramen ovale. At autopsy, we observed that this varied considerably between hypoxemic lambs. Shunting of microspheres across the foramen ovale during injection renders the calculation of blood flow to the lung based on the arterial reference sample meaningless. Second, bronchial arterial blood flow is increased in congenital cyanotic heart disease that is accompanied by a decreased pulmonary blood flow; in experimental conditions it increases within a week in the dog [102]. Third, the arteriovenous shunting of 15 µm microspheres was estimated to be between 2-9 % in rats, rabbits and pigs [45, 133, 192]. In conditions with increased arteriovenous shunting this number is higher [133]. Thus, these three pathways all may contribute to the increased number of microspheres in the lung in hypoxemic lambs. An increased bronchial arterial blood flow and an increased arteriovenous shunting are compatible with a lower nutritional blood flow to organs, while systemic blood flow is maintained.

Blood flow and oxygen supply to vital organs were unaltered in hypoxemic lambs. In another study we demonstrated that myocardial blood flow was increased in hypoxemic lambs to meet the increased demand for oxygen (Chapter 6). The present study demonstrates the extent of vasodilation in the coronary vascular bed, that is required to maintain myocardial blood flow in the presence of the increased whole blood viscosity.

Cerebral blood flow was unaltered in chronically hypoxemic lambs despite the fact that they were both hypocapnic and polycythemic, factors that have been shown to decrease cerebral blood flow. Hypocapnia decreases cerebral blood flow in fetal, newborn and adult sheep [178]. During 96 h of simulated high altitude in sheep, cerebral blood flow initially increased, but returned to control levels after 48-72 h when hypocapnia developed, whereas it remained increased in eucapnic sheep [126-128]. The effect of polycythemia on cerebral blood flow has been studied after isovolemic exchange transfusion with either oxyhemoglobin or methemoglobin containing packed red cells in newborn lambs [113, 147]. After exchange transfusion with oxyhemoglobin containing red cells, cerebral blood flow

decreased, but cerebral oxygen supply and oxygen uptake were unaltered. After exchange transfusion with methemoglobin containing red cells, cerebral blood flow decreased to a lesser extent than after exchange with oxyhemoglobin containing red cells, but cerebral oxygen supply decreased and oxygen uptake was maintained by increasing oxygen extraction. Similar results were obtained after increasing plasma viscosity by isovolemic exchange transfusion with dextran [85]. These results demonstrate that hypocapnia and an increased whole blood viscosity both may decrease cerebral blood flow and oxygen supply, but that cerebral oxygen uptake is maintained. In our study cerebral blood flow increased when arterial oxygen concentration decreased (Fig. 4.3) in a fashion quite similar to that previously reported [113, 147]. Moreover, the effect of increased whole blood viscosity on cerebral blood flow was clearly compensated for by a decrease in cerebral vascular hindrance (Fig. 4.3). Furthermore, our results suggest that the effect of hypocapnia on the cerebral vascular bed is operative, because cerebral vascular hindrance tended to increase with decreasing arterial P_{CO_2}. Thus, although the effects of increased whole blood viscosity and of hypocapnia on cerebral blood flow are clearly present in chronically hypoxemic lambs, we assume that they are overridden by vasodilating effects that are mediated by local metabolic factors.

Blood flow and oxygen supply to non-vital organs tended to be decreased in hypoxemic lambs, but we assume that this does not affect the oxygen uptake by these organs, because of their ample oxygen extraction reserve. Gastrointestinal blood flow and oxygen supply in hypoxemic lambs was 70 % of that in control lambs, a decrease which in acute experimental conditions does not lead to a decrease in gastrointestinal oxygen uptake [59]. The alterations in gastrointestinal blood flow and oxygen supply will also affect hepatic oxygen supply. Hepatic blood flow in hypoxemic lambs was 50 % lower than in control lambs. and the oxygen concentration of the portal venous blood, which comprises 90-95 % of hepatic blood flow in lambs, most likely is decreased in hypoxemic lambs. However, hepatic oxygen extraction can increase up to 80 % in lambs, so that oxygen uptake is maintained over a wide range of oxygen supply [58]. The decreased blood flow and oxygen supply to the kidney most likely does not interfere with adequate renal oxygen supply, because renal oxygen extraction is low [116].

The decrease in renal blood flow may affect renal function, because the increase in glomerular filtration rate (GFR) during development is mainly determined by renal plasma flow [3, 115]. In hypoxemic lambs in our study renal plasma flow was 50 % of that in control lambs. In another study we demonstrated that the GFR was similar in hypoxemic and in control lambs (2.7 mL/min·kg, Chapter 5) and comparable to values reported for 6-week-old lambs [3]. From the GFR and the renal plasma flow one can estimate that the filtration fraction in hypoxemic lambs (29 %) is twice as high as in control lambs (15 %). Because the

renal vascular hindrance was similar in hypoxemic and control lambs, we speculate that the GFR in hypoxemic lambs is maintained by an alteration in the balance between afferent and efferent arteriolar tone.

In conclusion, we demonstrated that blood flow and oxygen supply to vital organs is maintained in chronically hypoxemic lambs by adjustments in vascular tone, whereas blood flow to non-vital organs tends to be decreased as a consequence of the increased whole blood viscosity. The decreased oxygen supply to non-vital organs is presumably compensated for by an increase in oxygen extraction, so that oxygen uptake is maintained. However, the decreased perfusion of organs may limit the reserve to perform normal organ function. When extrapolating these results to patients with cyanotic heart disease, it must be taken into account that whole blood viscosity increases only moderately in hypoxemic lambs, because lambs have a low hemoglobin concentration. The effects of the increased whole blood viscosity on organ perfusion when hemoglobin concentration is higher will be more prominent.

5

Body fluid compartment volumes in chronically hypoxemic lambs

Congenital heart disease is often associated with a decreased growth rate, characterized by a decreased gain in body mass and length [135, 188, 212]. It seems to affect children with cyanotic heart disease more than children with non-cyanotic heart disease [135, 188, 212]. In young experimental animals exposed to various forms of chronic hypoxemia a decreased body mass is a common finding [62, 163, 176, 213, 221]. Based on studies in rats and mice, it has been suggested that a decreased rate of cell division is responsible for the growth retardation [62, 163].

The volumes of body fluid compartments change during growth. Both extracellular and intracellular water volumes increase, but per unit body mass total water and extracellular water volume decrease and intracellular water volume increases [40, 76]. This can in part be explained by a relatively faster growth of the intracellular compartment [40, 41]. In chronic hypoxemia, therefore, body fluid compartment volumes may be altered by the effects of decreased growth.

Another factor that may affect fluid compartment volumes is the effect of chronic hypoxemia on blood volume. Blood volume increases through an increase in red cell volume, while plasma volume is either normal or decreased [179, 230].

Fluid compartment volumes and blood volume have been measured in chronic hypoxemia. However, to our knowledge, the effect of cyanotic heart disease on fluid compartment volumes and on blood volume has not been measured simultaneously in young, growing subjects. Therefore, we measured total body water volume, extracellular water volume and plasma volume in lambs that had been hypoxemic for 3-4 weeks, due to experimental cyanotic heart disease and we calculated intracellular water volume, interstitial volume and blood volume. Thus, the relation between the lower body mass, that is found in these lambs [213], and the alterations in fluid compartment volumes, especially the intracellular water volume was studied, as well as the alterations in blood volume in relation to other fluid compartment volumes.

Materials and methods

Thirteen control lambs and 9 chronically hypoxemic lambs were studied. Eight control lambs and all hypoxemic lambs underwent surgery. Of these 8 control lambs, 4 were operated upon more than two weeks before the measurements, whereas the other 4 were operated upon approximately one week before the measurements. The other 5 control lambs stayed at a farm until a few days before the measurements and underwent neck vessel catheterization under local anesthesia, at least 24 h before the measurements. Statistical analysis was performed by one-way ANOVA to analyze the results of the three groups of control lambs and the hypoxemic lambs. When the F-value was higher than the critical value, multiple contrasts were used to test for the effects of surgery, timing of

surgery, and hypoxemia. Therefore, the following subgroups were compared: the two groups of control lambs that underwent surgery (n=4 in each group), control lambs that underwent surgery (n=8) versus those that underwent neck vessel catheterization (n=5), and control (n=13) versus hypoxemic lambs (n=9). In addition, the pooled data of all control lambs were compared with those of hypoxemic lambs by unpaired Student's t-test. For further details the reader is referred to Chapter 2.

Results

Age, body mass and fluid compartment volumes per unit mass in the 3 groups of control lambs and in hypoxemic lambs are shown in Table 5.1. Since no differences were found in any of these variables between the 3 groups of control lambs, the data of all control lambs were pooled.

Hypoxemic lambs were studied 26±3 days after the induction of hypoxemia. Their arterial oxygen saturation was decreased (65±11 *versus* 91±2 %, p<0.001) and their hemoglobin concentration was increased (142±16 *versus* 101±8 g/L, p<0.001).

Table 5.1 Fluid compartment volumes in control and hypoxemic lambs.

	Control Surgery (>2 w)	Control Surgery (<1 w)	Control No Surgery	Control Pooled Data	Chronic hypoxemia	t-test p-value
n	4	4	5	13	9	
Age (d)	43±7	40±7	37±2	40±5	37±4	
Days after surgery	22±10	6±1	4±2	10±9	29±3	
Body mass (kg)	13.2±2.4	12.0±2.4	13.6±3.7	13.0±2.8	10.5±2.3	<0.05
Total water (mL/kg)	765±22	791±58	784±40	780±40	752±27	
Extracellular water (mL/kg)	277±12	278±19	276±19	277±15	307±27	<0.01
Intracellular water (mL/kg)	488±16	512±49	503±36	501±35	445±27*	<0.001
Blood volume (mL/kg)	78±21	83±34	83±13	82±21	121±29*	<0.01
Red cell volume (mL/kg)	23±6	22±13	23±6	22±8	48±12*	<0.001
V_{ec}/V_{ic}	0.57±0.05	0.54±0.05	0.56±0.05	0.56±0.04	0.69±0.10*	<0.01

Control group is divided into lambs that underwent surgery (Control - Surgery) more than 2 weeks (>2 w) before the experiment, or approximately 1 week before (<1 w) the experiment, and lambs that underwent neck vessel catheterization (Control - No Surgery). In addition, pooled data for control lambs are shown (4th column), and the results of the unpaired t-test for all control (n=13) versus hypoxemic lambs (n=9). V_{ec}/V_{ic} = Extracellular to intracellular water ratio. * Hypoxemic lambs (n=9) significantly different from control lambs (n=13) by ANOVA and multiple contrasts, P<0.05.

Body mass of hypoxemic and control lambs and its division into compartments is shown in Fig. 5.1. Body mass and intracellular water volume were lower in hypoxemic lambs, while the extracellular water volume and the mass of solids were similar in hypoxemic and control lambs. The difference in body mass between hypoxemic and control lambs could almost completely be accounted for by the difference in total body water volume (7.9±1.8 *versus* 10.3±2.3 L, $p<0.02$, t-test).

Per unit body mass total body water was similar in hypoxemic and control lambs, but extracellular water volume was higher in hypoxemic lambs and intracellular water volume was lower (Table 5.1). V_{ec}/V_{ic}, the ratio of extracellular to intracellular water volume (Fig. 5.2), decreased with body mass in hypoxemic lambs ($y = -0.03x + 1.04$, $r = 0.81$, $p<0.01$), but did not change with body mass in control lambs ($y = 0.003x + 0.52$, $r = 0.10$). V_{ec}/V_{ic} was higher in hypoxemic than in control lambs (0.69±0.10 *versus* 0.55±0.04, $p<0.01$). Plasma osmolality (281±10 *versus* 286±4 mmol/L), total protein concentration (61±7 *versus* 59±5 g/L), sodium concentration (144±4 *versus* 145±2 mmol/L), and glomerular filtration rate (2.7±0.5 *versus* 2.7±0.4 mL/min·kg) were similar in hypoxemic and control lambs.

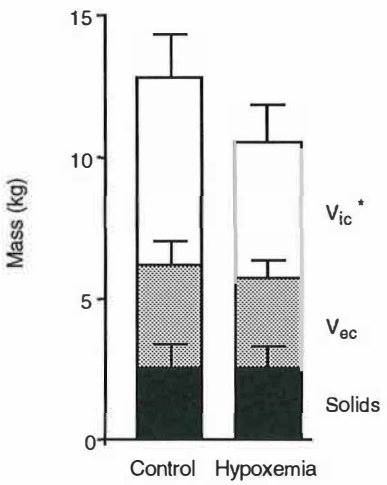

Figure 5.1 Body mass in control and hypoxemic lambs, and the division thereof in solids, and extracellular (V_{ec}), and intracellular (V_{ic}) fluid. Mean and SD for solids, V_{ec}, and V_{ic} are shown. * Hypoxemic lambs (n=9) significantly different from control lambs (n=13) by ANOVA and multiple contrasts, $P<0.05$.

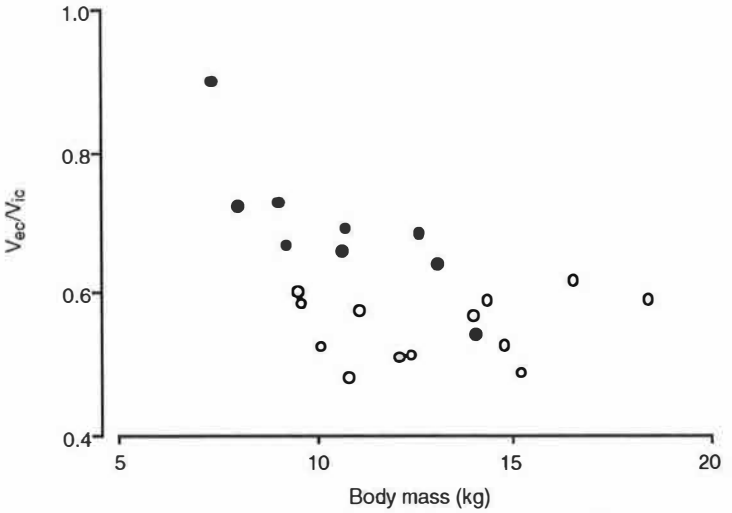

Figure 5.2 Extracellular to intracellular water volume ratio (V_{ec}/V_{ic}) as a function of body mass in control (open circles) and hypoxemic (solid circles) lambs.

Blood volume (Fig. 5.3) was increased in hypoxemic lambs, because of an increased total red cell volume. The total amount of hemoglobin in hypoxemic lambs was twofold increased (177±51 *versus* 97±28 g, $p<0.01$).

The intracellular water volume that was extravascular was lower in hypoxemic lambs (415±29 *versus* 489±34 mL/kg, $p<0.001$). $V_{int}/V_{ic}^{extravascular}$, which is the extravascular equivalent of V_{ec}/V_{ic}, was also increased in hypoxemic lambs (0.57±0.07 *versus* 0.45±0.05, $p<0.001$).

Discussion

In the present study we demonstrated that 3-4 weeks of chronic hypoxemia in lambs, due to experimentally induced cyanotic heart disease, has two distinct effects on body fluid compartment volumes. First, blood volume was increased mainly through an increased red cell mass. Second, intracellular water volume was decreased, which almost completely accounted for the difference in total body water volume and body mass between hypoxemic and control lambs. Since total water volume per unit of mass was similar in hypoxemic and control lambs, a larger fraction of body water was distributed to the extracellular space in hypoxemic lambs, which was demonstrated by the increased ratio of extracellular to intracellular water volume.

We found no differences between the 3 subgroups of control lambs (Table 5.1), indicating that surgery or the timing of surgery did not affect body fluid compartment volumes. Although the number of lambs in each subgroup is small,

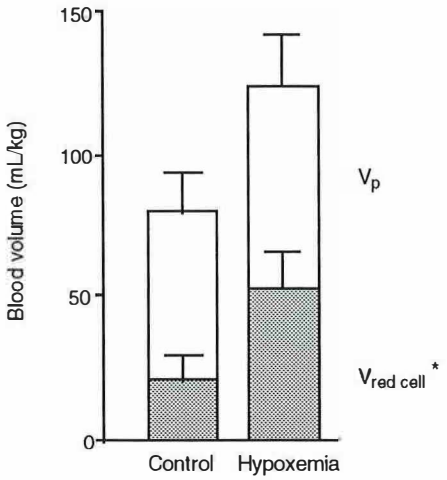

Figure 5.3 Blood volume and its components per unit mass in control and hypoxemic lambs. Mean and SD for plasma volume (V_p) and for red cell volume ($V_{red\ cell}$) are shown. * Hypoxemic lambs (n=9) significantly different from control lambs (n=13) by ANOVA and multiple contrasts, $P<0.05$.

the data of the control lambs are very similar to each other and in contrast with those of hypoxemic lambs. When a t-test is applied some more differences between control and hypoxemic lambs become apparent. For example, body mass is lower in hypoxemic lambs, which is in accordance with the decreased gain in body mass that has been shown in hypoxemic lambs by Teitel et al. [213].

Alterations in body fluid compartment volumes in (congenital) heart disease, may be related to water and salt retention [195, 232]. However, the alterations in fluid compartment volumes in the hypoxemic lambs in our study are not readily explained by this mechanism. Water and salt retention is associated with an increase in total body water and extracellular water volume, while intracellular water volume is not decreased [91]. In dogs with an experimental pulmonary stenosis no signs of venous congestion or water and salt retention were found [13, 14]. The hypoxemic lambs in our study had no signs of venous congestion either. Water and salt retention in chronic obstructive lung disease was related to hypercapnia rather than to hypoxemia [66, 190]. In the early phase of (simulated) high altitude hypoxemia, voluntary sodium and water intake are decreased and natriuresis and diuresis are increased [112]. In chronically hypoxemic rats glomerular filtration rate (GFR) and urinary sodium excretion were normal [169], similar to the GFR in our study. It is improbable that body water in hypoxemic lambs was redistributed due to osmotic shifts, because plasma osmolality, sodium and total protein concentrations were not increased. Thus, water and salt retention or an osmotic shift of water from the intracellular to the extracellular compartment cannot

explain the differences in fluid compartment volumes between hypoxemic and control lambs.

Fluid compartment volumes per unit body mass change during growth. After birth, total body water and extracellular water volume decrease and intracellular water volume increases [40, 76]. These changes have been attributed both to a relative loss of body water and to growth of the intracellular compartment. In infants, a redistribution of water from the extracellular to the intracellular compartment was found in the first two weeks of life and this was attributed to the effects of cellular growth [41]. In children, extracellular water volume decreases from 350 mL/kg to 280 mL/kg in the first six months of life and after 9-12 months extracellular water volume makes up a constant fraction of total body water, similar to that in adults [40]. Hence, from that time on the ratio of extracellular to intracellular water volume (V_{ec}/V_{ic}) will not change anymore. When growth is decreased, one may expect that extracellular water volume and V_{ec}/V_{ic} are increased, as compared with those of normally grown peers, and that adult values will be reached later, if at all. This is supported by the observation that in young rats on a low caloric or a low protein diet a decreased rate of cellular growth or cellular wasting was accompanied by an increased extracellular volume [69].

Similar changes in body fluid compartment volumes during growth as have been observed in children may be expected in lambs. Presumably, these changes in lambs occur over a shorter period of time, because they grow much faster. Newborn lambs double their body mass in 3 weeks and triple it in 7-8 weeks [138]. To the extent that total body water volume is a good indicator of lean body mass, our results indicate that it makes up a similar fraction of total body mass in hypoxemic and control lambs. However, the fact that V_{ec}/V_{ic} is increased in hypoxemic lambs, indicates that intracellular volume in hypoxemic lambs is a smaller fraction of lean body mass than in control lambs. The fact that their $V_{int}/V_{ic}^{extravascular}$, the extravascular equivalent of V_{ec}/V_{ic}, is also increased, indicates that the difference in V_{ec}/V_{ic} between hypoxemic and control lambs is not related to the changes in blood volume. In addition, V_{ec}/V_{ic} decreases with body mass in hypoxemic lambs, whereas it is stable over a broad range of body mass in control lambs. This suggests that V_{ec}/V_{ic} in control lambs has become stable and may resemble that of adult sheep, while in hypoxemic lambs it resembles that of younger, less mature lambs. These differences between hypoxemic and control lambs can be explained by assuming a decreased growth of the cellular compartment.

The mechanism of decreased growth in hypoxemia is unclear. In hypoxemic children no relation between decreased growth and either nutritional intake or the severity of hypoxemia has been found [135, 188, 212]. In experimental hypoxemic animals a decreased growth rate has been related to a decreased rate of cell

division rather than to a decreased cell volume [62, 163]. Both a decreased caloric intake and an effect of hypoxemia on cell division have been implicated as the cause of the decreased rate of cell division [62, 163]. The distinction between decreased cell numbers or decreased cell volume is important, because when cell numbers are decreased catch-up growth may not completely compensate for the growth retardation [236]. The results of our study, however, offer no clues about the mechanism of the decreased cellular growth in hypoxemia.

The second goal of our study was to determine the effects of chronic hypoxemia on blood volume and its components. Blood volume in hypoxemic lambs was increased, mainly through an increased red cell volume. This is consistently found in hypoxemia [168, 179, 189, 230]. The effects of hypoxemia on plasma volume, however, are variable. In acute hypoxemia plasma volume decreases [94, 112]. In chronic hypoxemia plasma volume is normal, but tends to decrease when hematocrit increases to over 60 % [179, 189, 230]. In rats exposed to simulated high-altitude, plasma volume decreased concurrent with an increase in both total blood volume and red cell volume, while hematocrit increased from 50 % at sea level to 75 % at high-altitude [168]. The mechanism behind the decrease in plasma volume is unclear. Since the fall in plasma volume prevents an excessive increase in blood volume it may be mediated through ANF release, as has been shown in conditions of increased intravascular volume [84]. A disadvantage of a decrease in plasma volume is a further increase of blood viscosity. Even though blood volume was increased by 50 % in our hypoxemic lambs, there were no signs of a decreased plasma volume or of increased vascular filling, suggesting that a mechanism to decrease the intravascular volume was not yet activated.

In conclusion, we have demonstrated that intracellular water volume is decreased in chronically hypoxemic lambs and that this is the main cause of their decreased body mass. The alterations in extracellular and intracellular water volumes per unit mass and in V_{ec}/V_{ic} can be explained by assuming a decreased growth of the cellular compartment. They are not related to the increase in blood volume, which is mainly brought about by an increase in the red cell volume. These results confirm and extend previous observations that the adaptation to chronic hypoxemia in lambs is established almost completely through an increase in hemoglobin concentration and at the expense of growth, by linking the decreased body mass to a decreased intracellular volume and by showing the extent of the erythropoietic response.

6

Left ventricular oxygen and substrate uptake in chronically hypoxemic lambs

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 hemoglobin concentration [6, 30, 65, 71, 110, 201, 202].

In chronic hypoxemia the increased hemoglobin concentration compensates for the decreased arterial oxygen saturation [213], 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 [159]. 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 as compared with control animals [31, 221], 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 will be met 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 [213]. 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 [90]. In children with cyanotic heart disease the activity of rate-limiting oxidative enzymes for carbohydrates and fatty acids were similar as in children with non-cyanotic heart disease [54], 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 [99]. Glucose supply and uptake by the myocardium was increased during infusion of catecholamines [149]. Catecholamine concentrations were either increased or normal in chronic hypoxemia [32, 119, 238, 245]. In hypoxemic adults, native to high altitude, myocardial lactate and pyruvate uptake was increased and this was linearly related to the increased arterial substrate concentrations [160]. Therefore, we hypothesized that glucose, pyruvate, lactate, free fatty acids 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 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 weeks of hypoxemia, and whether left ventricular oxygen uptake and supply are increased proportionally. Second, we wanted to determine whether glucose, pyruvate, lactate, free fatty acids 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

Fourteen control lambs and 15 chronically hypoxemic lambs were studied. All lambs underwent surgery. Statistical analysis was performed by unpaired Student's t-test and by multiple linear regression. For further details the reader is referred to Chapter 2.

Table 6.1 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*
Hemoglobin concentration (g/L)	102±11	139±16*
Arterial oxygen concentration (mL/L)	127±22	129±15
Oxygen uptake (mL/min·kg)	7.0±1.9	8.3±2.2*
Systemic blood flow (mL/min·kg)	145±49	182±73
Heart rate (beats/min)	118±25	166±33*
Systemic oxygen supply (mL/min·kg)	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*
PCO_2 (kPa)	5.2±0.6	4.3±0.7*
PO_2 (kPa)	13.3±1.8	7.9±1.3*
bicarbonate (mmol/L)	24.5±2.9	18.5±3.6*
Epinephrine (nmol/L) #	1.2±0.7	2.0±3.8
Norepinephrine (nmol/L) #	10.9±9.5	17.9±26.8

n=13 for control lambs, n=11 for hypoxemic lambs. * Hypoxemia significantly different from control by unpaired Student's t-test, $p<0.05$.

Results

General (Table 6.1). Hypoxemic lambs were slightly younger than control lambs and their body mass was lower. The arterial oxygen saturation was decreased and the hemoglobin concentration was increased in hypoxemic lambs, so that 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.

Heart. Myocardial mass was increased in hypoxemic lambs, mainly because of an increased right ventricular mass (Fig. 6.1). Total myocardial blood flow was increased in hypoxemic lambs (102±30 *versus* 56±24 mL/min, $p<0.001$). Per 100g 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. 6.2).

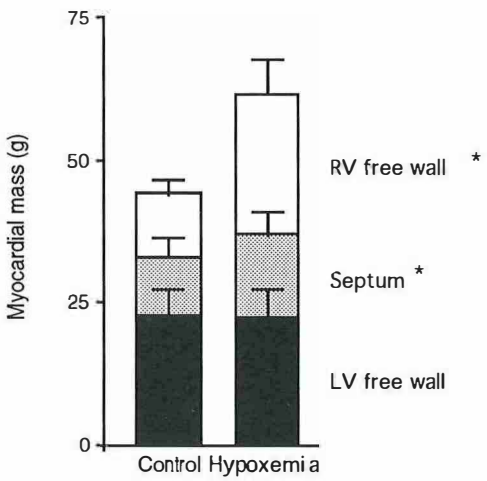

Figure 6.1 Myocardial mass and its subdivision into left and right ventricular free wall, and septum in control and in hypoxemic lambs. Mean and SD for each ventricular part is shown. * $P<0.05$.

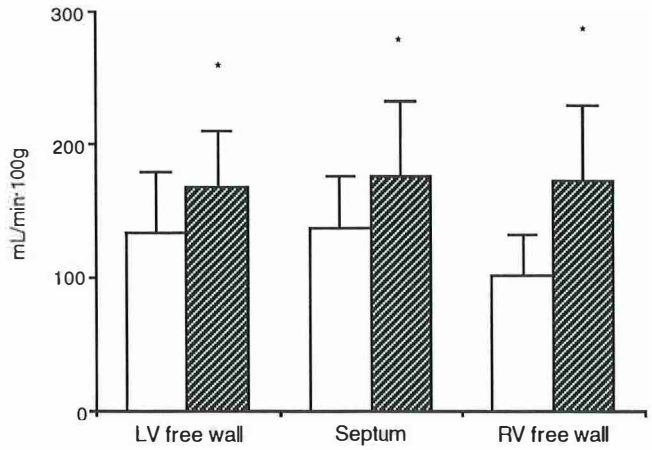

Figure 6.2 Blood flow to the ventricular myocardium per unit mass in control lambs (open bars) and in hypoxemic lambs (hatched bars). * P<0.05.

Left ventricular resistance was similar in hypoxemic and control lambs (0.06±0.02 *versus* 0.08±0.02 kPa·min·100g/mL). The coronary sinus blood in hypoxemic lambs had a lower oxygen saturation (16±6 *versus* 25±10 %, p<0.001) and P_{O_2} (3.7±1.1 *versus* 4.6±0.8 kPa, p<0.05) than in control lambs. Consequently, the arterio-coronary sinus concentration difference for oxygen was maintained at similar levels in hypoxemic and control lambs (4.3±0.8 *versus* 4.1±0.8 mmol/L).

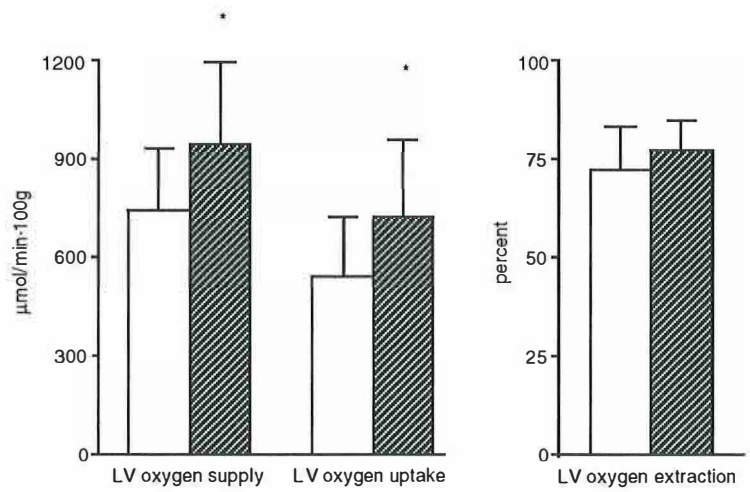

Figure 6.3 Left ventricular oxygen supply (left panel), oxygen uptake (middle panel), and oxygen extraction (right panel) in control (open bars) and in hypoxemic lambs (hatched bars). * P<0.05.

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. 6.3). Left ventricular oxygen uptake per beat was similar in hypoxemic and control lambs (4.5±1.9 *versus* 4.5±1.0 μmol/100g). The rate

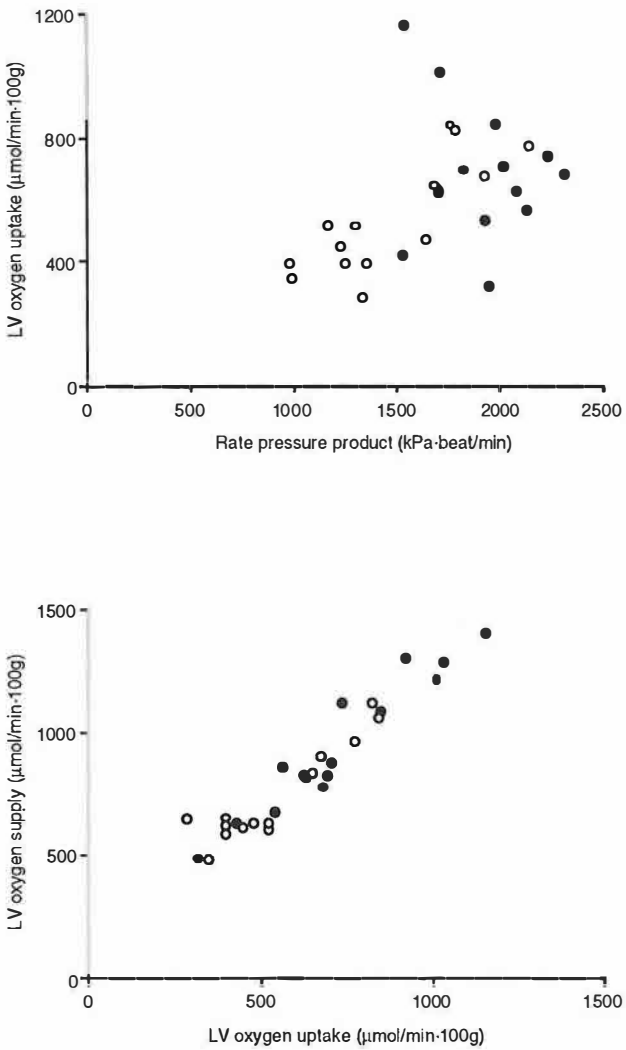

Figure 6.4 Upper panel: left ventricular oxygen demand, estimated by the rate pressure product, versus left ventricular oxygen uptake in control (open dots) and in hypoxemic lambs (solid dots); regression for control and hypoxemic lambs $y = 0.26x + 173$, $r = 0.59$, $p<0.001$. Lower panel: left ventricular oxygen uptake versus left ventricular oxygen supply in control (open dots) and in hypoxemic lambs (solid dots); regression for control and hypoxemic lambs $y = 1.08x + 158$, $r = 0.96$, $p<0.001$

pressure product was increased in hypoxemic lambs (2072±465 *versus* 1467±358 kPa·beat/min, $p<0.001$). There was a linear relation between the rate pressure product and the left ventricular oxygen uptake and hypoxemia had no effect on this relationship (Fig. 6.4). 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. 6.4) 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. 6.5). 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 of acetoacetate was slightly different in hypoxemic as compared with control lambs, whereas for the other substrates no differences were found (Fig. 6.5); there was no net lactate production. The OERs, however, indicate that the contribution of pyruvate and acetoacetate to left ventricular substrate uptake was only small (Table 6.2). Free fatty acids and β-hydroxybutyrate were the most important fuels for the left ventricle that we identified, whereas the contribution of glucose, pyruvate and lactate was only small both in 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.

Table 6.2 Oxygen extraction ratios.

	Control	Hypoxemia
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
Free fatty acids #	0.28±0.38	0.19±0.32
Total #	0.51±0.50	0.45±0.43

n=11 for hypoxemic lambs.

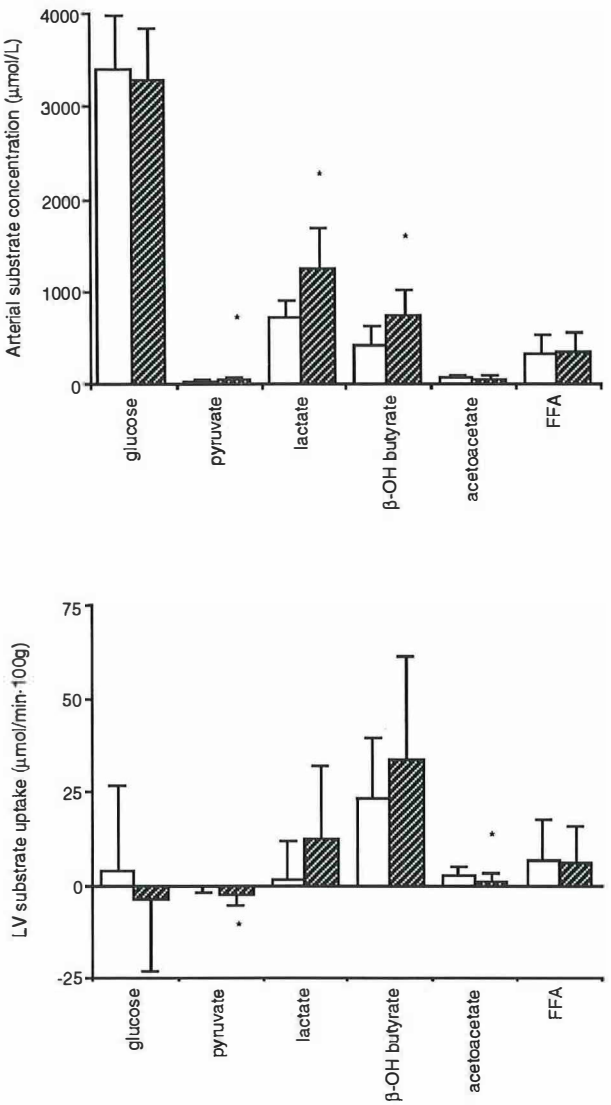

Figure 6.5 Arterial substrate concentration (upper panel) and left ventricular substrate uptake (lower panel) in control lambs (open bars) and in hypoxemic lambs (hatched bars). * $P<0.05$.

Discussion

In the present study we demonstrated that, in lambs after 4 weeks of hypoxemia, left ventricular oxygen supply and oxygen uptake were proportionally increased to meet the increased oxygen demand of the left ventricle. The increase in oxygen demand and oxygen uptake was directly related to the increased heart rate in hypoxemic lambs. We also demonstrated that left ventricular substrate uptake was practically unaltered in hypoxemic lambs as 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 was not increased. In addition, glucose, pyruvate, and lactate uptake was negligible, and free fatty acids 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·100g), but gradually decreases to approximately 50% of this value in adult sheep [73, 74]. This is related to a decrease in left ventricular oxygen uptake per unit mass and an increase in hemoglobin concentration [73, 74, 138]. 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 left ventricular oxygen supply matched to oxygen uptake (Fig. 6.4). It is readily apparent from Figure 6.4 that the matching of left ventricular oxygen supply to oxygen uptake is unaltered in hypoxemic lambs as 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 similar fashion as 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 [5]. However, the increase in right ventricular oxygen supply, in these conditions, may be out of proportion to the increase in oxygen demand [5]. Thus, the twofold 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 under various (patho)-physiological conditions, because the oxygen extraction reserve of the heart is

limited. Myocardial blood flow is increased in order to increase oxygen supply in proportion to oxygen uptake [218] or to maintain oxygen supply during (severe) acute hypoxemia or anemia [6, 30, 65, 110, 201]. Conversely, myocardial blood flow decreases during acute polycythemia [6, 65, 110]. 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 [65]. Similarly, maximal myocardial oxygen supply decreased during progressive polycythemia in dogs, indicating that the viscosity of blood increased relatively more than the oxygen capacity [6]. In another set of experiments, we found that whole blood viscosity in hypoxemic lambs was increased as compared with control lambs (4.4 *versus* 3.6 mPa.s, shear rate 100 s^{-1}, Chapter 4). The difference in vascular tone in these conditions can be estimated by the ratio of resistance and whole blood viscosity [65]. Because left ventricular resistance in hypoxemic lambs was approximately 0.8 of that in control lambs, and whole blood viscosity 1.25 of that in control lambs, this ratio was approximately 0.7 (=0.8/1.25), 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 [57, 153]. In all studies an increased capillary density is found in the right ventricle [43, 145, 153, 220]. 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 [153], whereas in other studies no effect of hypoxemia on the capillary density of the left ventricle was observed [43, 145, 220]. 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 6.1). 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 as

compared with those in non-cyanotic subjects [103], 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 (Chapter 4) [31].

Left ventricular substrate uptake in hypoxemic lambs was practically unaltered as compared with control lambs, and free fatty acids and β-hydroxybutyrate were important fuels, whereas the uptake of glucose, pyruvate and lactate was negligible. Similar results have been obtained in 6-week-old lambs with and without an aortopulmonary left-to-right shunt in our laboratory [92]. A low myocardial glucose, pyruvate and lactate uptake has also been found in newborn lambs and adult sheep [73, 74]. In contrast, myocardial lactate and pyruvate uptake increased linearly with arterial substrate concentration in chronically hypoxemic adults, native to high altitude [160]. In that study, myocardial oxygen uptake was decreased, which was related to an increased efficiency [159]. 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, pyruvate and in our lambs is compatible with a postabsorptive state [166]. In these conditions the breakdown of glycogen, as well as the utilization of glucose through the glycolytic pathway is inhibited [164], so that glucose utilization from endogenous stores is unlikely. The triglyceride pool is another source of endogenous substrate. Zierler suggested that all fatty acids taken up by the myocardium are initially stored in a pool and are utilized from this pool [247]. 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 [237]. In anesthetized dogs virtually all of the ^{14}C-labeled palmitate taken up by the myocardium underwent rapid oxidation [162] and the oxidation of glucose, lactate and fatty acid uptake accounted for all of the oxidative metabolism in resting conditions [211, 237]. These results suggest that even if all free fatty acids taken up by the myocardium are initially stored in a pool, their utilization 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 one. In another study from our laboratory we demonstrated that triglycerides also contributed to left ventricular substrate uptake [90]. In addition, in sheep large amounts of short chain fatty acids are produced in the rumen, of which

acetate is largely delivered to peripheral tissues [24]. In resting skeletal muscle oxidation of acetate accounted for ± 20 % of the oxygen uptake, while during exercise this decreased to ± 5 % [117]. 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 as compared with normoxemia. Left ventricular substrate uptake in chronically hypoxemic lambs is practically unaltered as compared with control lambs. Free fatty acids and β-hydroxybutyrate being the most important substrates taken up by the myocardium that we identified, but we speculate that triglycerides and acetate may also contribute to left ventricular substrate uptake.

7

Cardiovascular adjustments to acute hypoxemia superimposed on chronic hypoxemia in lambs

Acute hypoxemia induces cardiorespiratory responses in order to maintain an adequate oxygen supply to the tissues. Heart rate, systemic blood flow, and ventilation all increase, while vascular resistance decreases, and systemic blood flow is redistributed [55, 123, 161, 201]. These adjustments are the result of local vascular, humoral and chemoreceptor responses [106]. Local vascular responses redistribute blood flow to metabolically active organs [106], adrenergic mechanisms increase heart rate and systemic blood flow [55, 202], and chemoreceptor stimulation induces an increase in ventilation and heart rate, and vasodilation [121, 186].

In chronic hypoxemia other adjustments come into play. Hemoglobin concentration, ventilation and heart rate are increased, while systemic blood flow is not increased [8, 49, 213]; it is, however, redistributed away from non-vital organs [31]. Adrenergic mechanisms are less important in chronic hypoxemia: catecholamine concentrations are not uniformly reported to be increased and adrenergic receptors are down-regulated [32, 49, 119]. Although chemoreceptor activity may be significant in chronic hypoxemia [234], the hypoxic sensitivity of peripheral chemoreceptors decreases after prolonged hypoxemia [34, 209, 234]. Thus, the cardiovascular responses to acute hypoxemia that are mediated through chemoreceptor or adrenergic stimulation may be blunted, when acute hypoxemia is superimposed on chronic hypoxemia.

Another factor that may affect the cardiovascular response to acute hypoxemia is the adequacy of myocardial oxygen supply. In another study we demonstrated that in chronically hypoxemic lambs left ventricular oxygen supply was matched to oxygen demand at the expense of the coronary flow reserve (Chapter 6). When acute hypoxemia is induced in normoxemic animals left ventricular blood flow, oxygen supply and oxygen uptake increase and no signs of myocardial oxygen lack are found, even during severe reductions in arterial oxygen saturation [55, 70, 72, 201, 202], so that one may expect that myocardial oxygen supply can be well maintained during mild to moderate acute hypoxemia superimposed on chronic hypoxemia.

Therefore, we studied the adequacy of the cardiovascular responses and of left ventricular oxygen supply during acute hypoxemia superimposed on chronic hypoxemia, induced by acutely increasing the cardiac right-to-left shunt. We hypothesized that the cardiovascular responses to acute hypoxemia might be blunted, but that left ventricular oxygen supply would be adequate.

Materials and methods

Nine chronically hypoxemic lambs were studied during steady state chronic hypoxemia and during acute hypoxemia superimposed on chronic hypoxemia. Each lamb served as its own control. Statistical analysis was performed by paired Student's t-test. For further details the reader is referred to Chapter 2.

Results

The lambs were 40±3 days old, weighed 11.7±3.4 kg and had an arterial oxygen saturation of 65±7 %. Determinants of systemic oxygen supply and oxygen uptake and their alterations during acute hypoxemia are shown in Fig. 7.1. The arterial oxygen concentration decreased by 37±10 % ($p<0.001$), while systemic blood flow increased (41±33 %, $p<0.01$), through statistically non-significant increases of both heart rate (148±25 *to* 182±39 beats/min, $0.05<p<0.10$) and left ventricular stroke volume (1.2±0.6 *to* 1.3±0.5 ml/kg), so that systemic oxygen supply was unaltered. Oxygen uptake was unchanged, because the mixed-venous oxygen saturation

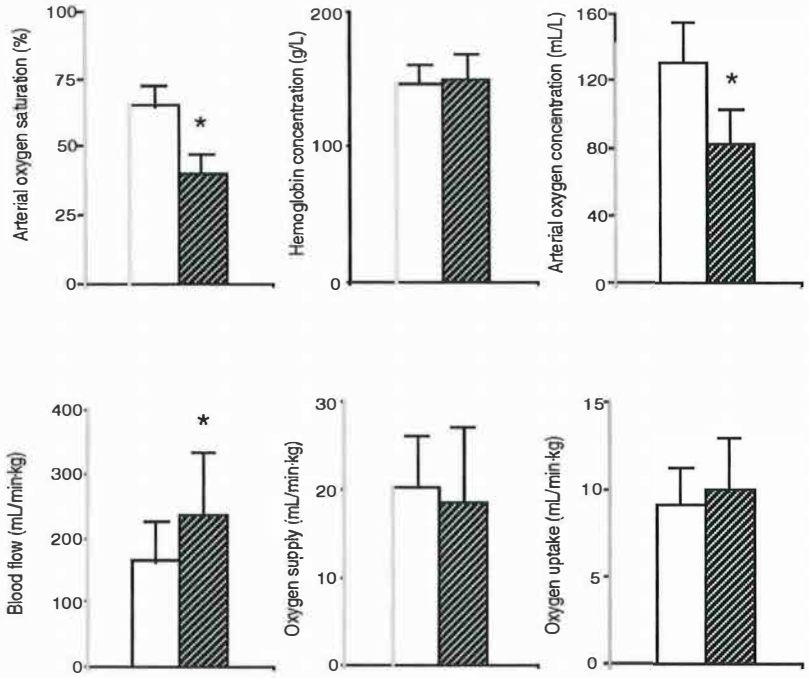

Figure 7.1 Systemic oxygen supply and its determinants, and oxygen uptake in lambs during chronic hypoxemia (open bars), and during acute hypoxemia superimposed on chronic hypoxemia (hatched bars). * $p<0.05$ by paired t-test.

Table 7.1 Blood pressures, vascular resistance, and blood gases.

	Control	Acute Hypoxemia
Blood pressures (mmHg)		
Arterial		
systolic	93±7	87±18
diastolic	65±11	65±14
mean	76±10	73±11
Right atrial (mean)	4±4	3±4
Left atrial (mean)	4±6	3±4
Systemic resistance (mmHg·min·100g/mL)	4.9±2.1	3.6±2.0*
Rate pressure product (mmHg·beat/min)	13744±1951	16003±5029

Right atrial pressures were obtained in 7 lambs, in the other lambs it was assumed to be 4 mmHg. * Chronic hypoxemia significantly different from superimposed acute hypoxemia by paired t-test, $p<0.05$.

decreased (35±5 *to* 18±6 %, $p<0.001$) and the oxygen extraction increased (45±5 *to* 56±8 %, $p<0.05$). Blood pressures were unaltered after the induction of acute hypoxemia, but systemic resistance decreased (Table 7.1). There were no signs of metabolic acidosis during acute hypoxemia, the arterial lactate concentration increased twofold, but the increase was not statistically significant (Table 7.2).

Table 7.2 Arterial and coronary sinus blood gases and lactate concentrations flow ratios.

	Control	Acute Hypoxemia
Blood Gases and pH		
Arterial		
pH	7.38±0.04	7.37±0.05
P_{CO_2} (kPa)	4.7±0.6	4.4±0.6*
P_{O_2} (kPa)	7.4±1.1	5.1±0.8*
bicarbonate (mmol/L)	19.8±3.6	18.5±3.1
Coronary Sinus		
pH	7.34±0.02	7.35±0.04
P_{CO_2} (kPa)	5.8±0.2	5.3±0.8
P_{O_2} (kPa)	3.5±1.1	2.8±0.6*
bicarbonate (mmol/L)	23.5±3.4	20.5±3.6
Lactate (µmol/L)		
Arterial	1315±192	2701±927
Coronary Sinus	1071±268	2560±850
Arterial - Coronary Sinus	244±162	142±122

* Chronic hypoxemia significantly different from superimposed acute hypoxemia by paired t-test, $p<0.05$.

Myocardial and adrenal blood flows increased during acute hypoxemia, cerebral blood flow also tended to increase (28±36 %, $0.05<p<0.10$), whereas blood flows to other organs did not change (Fig. 7.2). Endocardial to epicardial blood flow ratios decreased for both the left ventricular free wall (1.36±0.17 *to* 1.24±0.12, $p<0.05$)

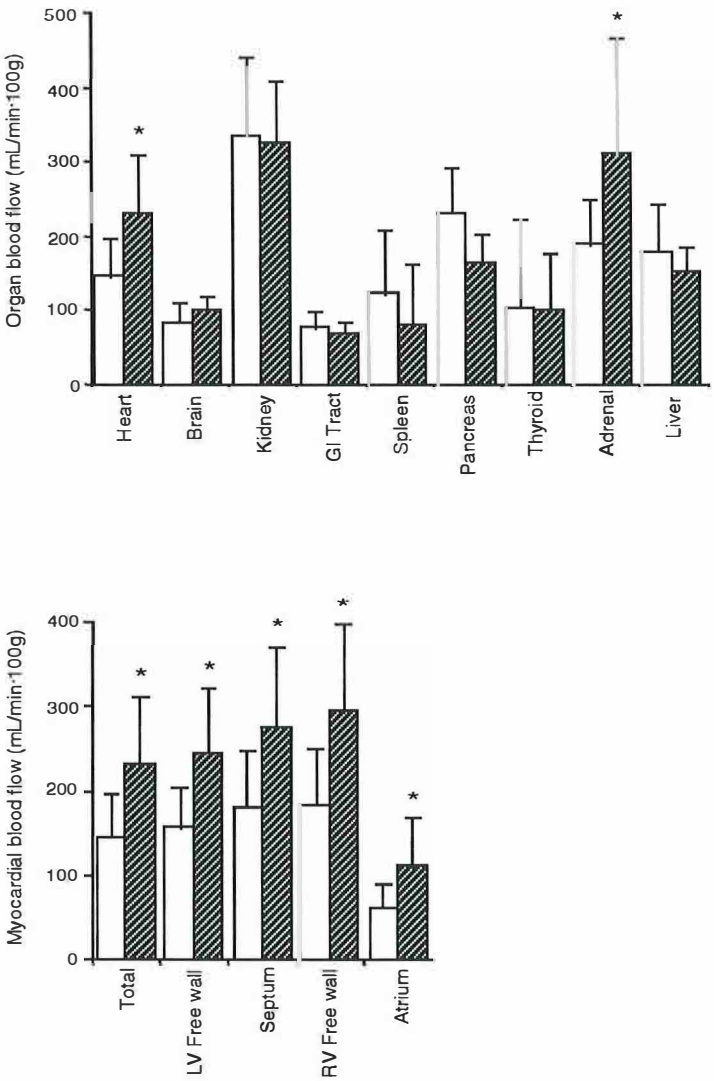

Figure 7.2 Blood flow to organs (upper panel) and to myocardial parts (lower panel) in lambs during chronic hypoxemia (open bars), and during acute hypoxemia superimposed on chronic hypoxemia (hatched bars). GI tract = gastrointestinal tract. n=7 for GI tract, liver, spleen and pancreas. * $p<0.05$ by paired t-test.

and the right ventricular free wall (1.34±0.10 *to* 1.17±0.14, p<0.001).
The alterations in organ blood flows corresponded to opposite changes in vascular resistance (Fig. 7.3): myocardial, cerebral, adrenal and total resistance decreased significantly. Myocardial, cerebral, adrenal and systemic oxygen supply were maintained during acute hypoxemia, whereas to most other organs it decreased (Fig. 7.3).

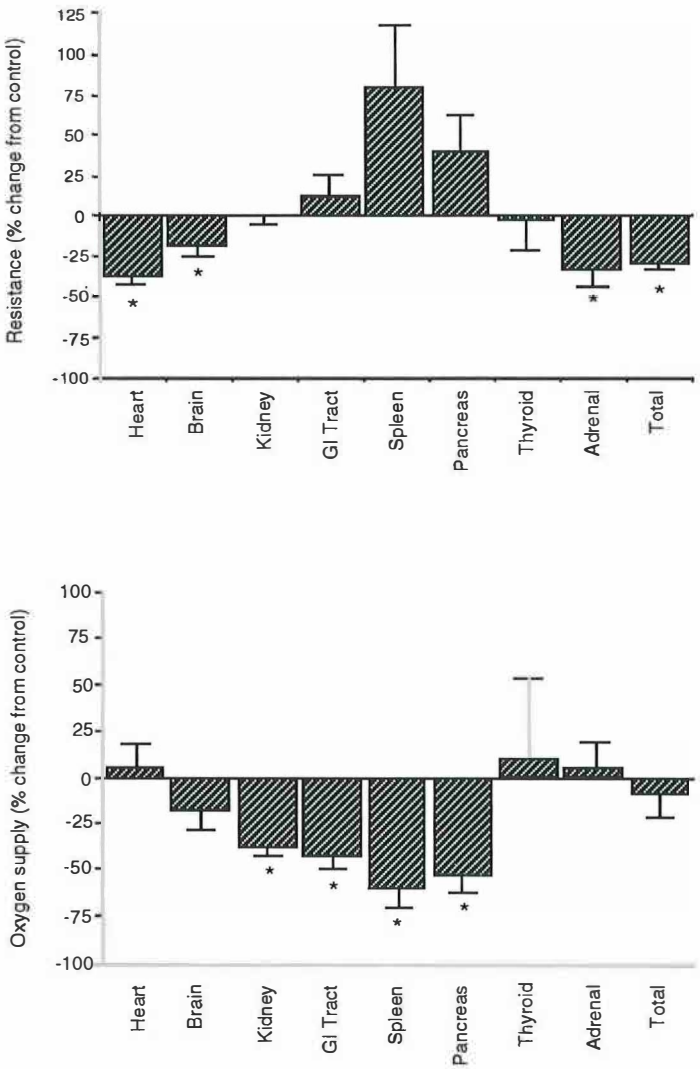

Figure 7.3 Percentage change in resistance (upper panel) and in oxygen supply (lower panel) during acute hypoxemia superimposed on chronic hypoxemia. GI tract = gastrointestinal tract. n=8 for the brain, n=7 for GI tract, liver, spleen and pancreas. * p<0.05 by paired t-test.

Cerebral oxygen supply ranged from 7.3 - 18.4 mL/min·100g during chronic hypoxemia and was somewhat lower during superimposed acute hypoxemia, but the difference was not statistically significant (Fig. 7.3). Cerebral oxygen supply was maintained during superimposed acute hypoxemia when cerebral oxygen supply during chronic hypoxemia was 10 mL/min·100g or lower. In contrast, it decreased when cerebral oxygen supply during chronic hypoxemia was higher than 10 mL/min·100g. The decrease in cerebral oxygen supply during superimposed acute hypoxemia was significantly related to the cerebral oxygen supply before the onset of acute hypoxemia (Fig. 7.4). These results indicate that cerebral oxygen supply decreased during superimposed acute hypoxemia in the lambs with the most luxurious cerebral oxygen supply, whereas it was maintained in the other lambs.

Coronary sinus oxygen saturation decreased (16±6 *to* 11±5%, $p<0.001$) and the arterio-coronary sinus oxygen concentration difference decreased by 37 % ($p<0.001$). However, the increase in left ventricular blood flow (Fig. 7.2) was sufficient to maintain oxygen supply to and oxygen uptake by the left ventricular free wall (747±255 *versus* 636±168 µmol/min·100g). The rate pressure product during acute hypoxemia was not significantly different from that during chronic hypoxemia (Table 7.1). There was no net lactate production by the myocardium during acute hypoxemia and coronary sinus blood gases showed no signs of metabolic acidosis (Table 7.2).

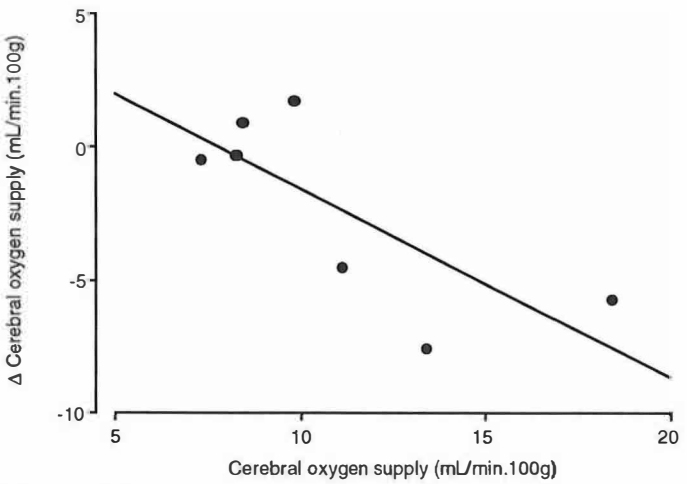

Figure 7.4 Change in cerebral oxygen supply during superimposed acute hypoxemia (ΔCerebral oxygen supply) as a function of cerebral oxygen supply before the induction of acute hypoxemia. The cerebral oxygen supply during superimposed acute hypoxemia decreased significantly more when the cerebral oxygen supply before the induction of acute hypoxemia was higher: regression $y=4.4 - 0.64x$, ($r^2=0.51$, $p<0.05$), $n=8$.

Discussion

In this study we demonstrated that acute hypoxemia superimposed on 3-4 weeks of chronic hypoxemia leads to an increase in systemic blood flow and a redistribution of blood flow towards vital organs. Consequently, systemic and myocardial blood flow is increased, so that systemic and myocardial oxygen supply and oxygen uptake are maintained. In addition, our results strongly suggest that the cardiovascular responses to superimposed acute hypoxemia are adequate to maintain cerebral oxygen supply at a sufficient level, although it decreases in those lambs with a more luxurious cerebral oxygen supply.

The cardiovascular adjustments to acute hypoxemia superimposed on chronic hypoxemia in our lambs are quite similar to those described by Sidi *et al.* for acute hypoxemia in 6-week-old normoxemic lambs [201]. In that study the arterial oxygen saturation decreased to 45%, similar to the level that was reached in our study, and heart rate and systemic blood flow increased to similar levels as in our lambs. In addition, the pattern of blood flow distribution and the alterations in local vascular resistances in that study were also similar to those in our lambs [201].

When acute hypoxemia is induced in normoxemic animals blood flow to vital organs increases, whereas blood flow to most non-vital organs is maintained [1, 55, 201]. Consequently, oxygen supply to vital organs is maintained or increases, whereas to non-vital organs it decreases. In chronic hypoxemia blood flow is also redistributed: blood flow to vital organs is maintained, whereas blood flow to non-vital organs is decreased as compared with that in normoxemic control lambs [31]. We found that this redistribution of blood flow is a consequence of the increased whole blood viscosity, due to the increased hemoglobin concentration in combination with vasodilation in vital organs (Chapter 4). The alterations in blood flow, resistance and oxygen supply of vital organs during acute hypoxemia in our study, suggest that the local vascular responses are unimpaired when acute hypoxemia is superimposed on 3-4 weeks of chronic hypoxemia.

Adrenal blood flow increases during acute hypoxemia [30, 55, 124] and epinephrine turnover increases as well [119]. The adrenergic response may contribute to the increase in heart rate and systemic blood flow: β-adrenergic blockade during acute hypoxemia abolished the increase in heart rate, systemic blood flow and oxygen uptake in newborn lambs [202]. In contrast, combined α- and β-adrenergic blockade during acute hypoxemia in adult dogs hardly affected the increase in heart rate and in systemic blood flow [55]. In our study adrenal blood flow increased during acute hypoxemia, suggesting adrenergic stimulation. In chronically hypoxemic lambs left ventricular β-adrenergic receptor density was decreased [32], but the maximal heart rate during catecholamine infusion was similar to that in normoxemic lambs [28]. These results suggest that the

cardiovascular response to adrenergic stimulation during acute hypoxemia in chronically hypoxemic lambs is unimpaired.

The ventilatory response to acute hypoxemia in the lambs in our study seemed blunted, because their arterial P_{CO_2} hardly decreased, whereas under normal conditions hypocapnic alkalosis develops during acute hypoxemia [55, 201]. However, after the induction of acute hypoxemia, our lambs demonstrated deeper breathing and nasal flaring, suggesting an increased ventilation. This discrepancy may be related to our experimental set-up. In most studies, hypoxemia is induced by lowering the inspired oxygen concentration, but in our study hypoxemia was induced by (further) increasing the cardiac right-to-left shunt. We assume that the increase in cardiac right-to-left shunt prevented an appreciable fall in arterial P_{CO_2}, despite a decrease of the alveolar P_{CO_2}. Similar observations have been made in exercising humans with a cardiac right-to-left shunt [203]. Thus, although only mild hypocapnia developed in the lambs in our study, it does not exclude an adequate ventilatory response. However, taking our experimental set-up into account, no meaningful evaluation of the ventilatory response is possible by using the alterations in the arterial blood gases.

One of the hypothesis underlying this study was that chronic hypoxemia attenuates the chemoreceptor-mediated response to acute hypoxemia, because of a decreased hypoxic sensitivity [34, 209, 210, 234]. The adequacy of the cardiovascular responses to superimposed acute hypoxemia in chronically hypoxemic lambs, suggest that their chemoreceptor-mediated responses were intact as well. There are several explanations why the hypoxic sensitivity of the chemoreceptors was unimpaired in the lambs in our study. First, hypoxemia had not been present from birth, but was induced between the 10th and 14th day of life. By that time chemoreceptors have been reset in lambs [36], and subsequent exposure to hypoxemia may have a different effect on the development of hypoxic sensitivity and its reversibility, than if hypoxemia has been present from birth [60, 101]. Second, a decrease in hypoxic sensitivity may take longer to develop than 3 to 4 weeks. Although our chronically hypoxemic lambs had an adequate response to acute hypoxemia, we are not certain that this would have been similar when the period of hypoxemia would have been longer than 3-4 weeks.

Left ventricular blood flow was increased during acute hypoxemia superimposed on chronic hypoxemia, so that left ventricular oxygen supply and oxygen uptake were maintained at the same level as during chronic hypoxemia. However, during acute hypoxemia in normoxemic lambs, resulting in a decrease in the arterial oxygen saturation and oxygen concentration comparable to that in the lambs in our study, left ventricular blood flow, oxygen supply and oxygen uptake all increased [70, 201, 202], indicating an increased oxygen demand. This raises the question whether left ventricular oxygen demand was met during acute hypoxemia

in our lambs. Because left ventricular contractility increases during acute hypoxemia [55, 70] and heart rate was slightly increased in our lambs, one would expect that left ventricular oxygen demand was increased as well. However, the rate pressure product was not significantly increased during acute hypoxemia in our study. Moreover, we found no evidence of left ventricular oxygen lack: there was no net lactate production and there was no metabolic acidosis in the coronary sinus blood during acute hypoxemia. Even though this does not exclude regional differences in lactate production and uptake [4, 148], it suggests that global left ventricular oxygen demand was met. Endocardial to epicardial blood flow ratio decreased after the induction of hypoxemia, but the perfusion of the endocardium still was higher than that of the epicardium, also suggesting an adequate perfusion of the most vulnerable part of the myocardium.

In another study we demonstrated that left ventricular blood flow in chronically hypoxemic lambs was somewhat increased to meet the increased demand for oxygen (Chapter 6). We suggested that this was established by some coronary vasodilation and at the expense of the coronary flow reserve, to allow for the increase in blood flow and to compensate for the increased whole blood viscosity. The coronary vascular tone in chronically hypoxemic lambs was estimated to be approximately 70 % of that in normoxemic lambs. The increase of left ventricular blood flow during acute hypoxemia in this study requires a further decrease of the coronary vascular tone to approximately 35 % of that in normoxemic lambs. Similar decrements in coronary vascular tone, to allow a threefold or higher increase in left ventricular blood flow, have been found during acute hypoxemia in lambs both before and after birth [70, 170, 201, 202]. Thus, although left ventricular oxygen uptake did not increase during acute hypoxemia, our results suggest that left ventricular oxygen demand was met during acute hypoxemia.

To what extent our results can be applied to acute hypoxemia induced by hypoxic spells in children with cyanotic congenital heart disease is uncertain. Several pathophysiologic mechanisms have been proposed to explain these spells. Increased right-to-left shunting, secondary to increased right ventricular outflow tract obstruction or increased pulmonary vascular resistance [111], a sudden increase of right-to-left shunt secondary to a Valsava maneuver or its equivalent and hyperpnea [96], and a decrease of systemic vascular resistance [79] all have been suggested as the primary event provoking such a spell. In our experimental set-up the primary event was an increase in right ventricular outflow tract obstruction, which is also true for most of the above suggested mechanisms. In that respect, the cardiovascular responses that we observed during acute hypoxemia superimposed on chronic hypoxemia in lambs may be quite similar to the responses during a hypoxic spell. However, if a decrease in systemic vascular resistance is the primary event provoking a hypoxic spell, the alterations in local

vascular resistances governing the redistribution of blood flow during acute hypoxemia, may be different than in our experiment.

In summary, we demonstrated that the cardiovascular responses to acute hypoxemia superimposed on 3 - 4 weeks of chronic hypoxemia in lambs were similar to those that have previously been described for normoxemic lambs. In addition, we demonstrated that left ventricular oxygen supply during acute hypoxemia was adequate to meet left ventricular oxygen demand. Thus, the cardiovascular response to acute hypoxemia superimposed on 3-4 weeks of chronic hypoxemia in lambs was adequate, so that the oxygenation of vital organs could be maintained in these conditions.

8

General discussion

We studied chronic hypoxemia in lambs with an experimentally induced atrial septal defect and pulmonary stenosis as first described by Teitel et al. [213]. Many aspects of the adjustments to chronic hypoxemia have been studied in acute experimental conditions, including acute hypoxemia and acute polycythemia. These studies provide a wealth of information about adjustments to hypoxemia and its consequences, but they do not take the long-term effects of chronic hypoxemia into account. We studied lambs after 3-4 weeks of hypoxemia allowing the adjustments to reach a stable level. Measurement of systemic cardiovascular and hematological variables and growth, which can relatively easy be obtained in humans as well, was combined with the measurement of fluid compartments, organ blood flows, and myocardial metabolism at rest and during acute stress. This way the insight into the pathophysiology of chronic hypoxemia is further expanded, while at the same time a meaningful comparison with the studies in humans and in other (experimental) animals can be made.

Methodological considerations

Chronic hypoxemia has been studied in man and animals by exposing them to (simulated) high altitude. Many of these studies were performed in adult animals and focused on hematological, myocardial, or pulmonary adjustments. The (simulated) high altitude studies are considered in the next section, inasmuch as they are pertinent to this study.

Few investigators have experimentally induced a cardiac right-to-left shunt to simulate chronic hypoxemia in order to study congenital cyanotic heart disease. In adult dogs a cardiac right-to-left shunt has been created by connecting the inferior vena cava or the pulmonary artery with the left atrium. In these studies hematological, myocardial, and long-term ventilatory adjustments have been studied. However, not much attention has been paid to the consequences of chronic hypoxemia in young developing animals.

There are several reasons why we used lambs to study the effects of chronic hypoxemia. Lambs have a body mass at birth that is similar to that of infants. The development of cardiovascular and hematological variables and growth has been well characterized in lambs [138]. Because the blood volume is sufficiently large, blood samples can be taken repeatedly without inducing the effects of hemorrhage. In addition, the lamb has been widely used in pediatric research to study a wide range of acute and chronic stresses, so that reference data are available. In our laboratory we have considerable experience with the lamb as an experimental animal [89, 217].

It was our purpose to study hypoxemic lambs when the circulatory and hematological adjustments to hypoxemia were well stabilized. In addition, we wanted to be certain that the levels of fetal hemoglobin would be low. Teitel et al. demonstrated that after the induction of hypoxemia the hemoglobin concentration increased throughout a period of two weeks [213]. We studied 5- to 6-week-old lambs, after 3-4 weeks of hypoxemia, and found that the hemoglobin concentration was relatively stable in the last week before the measurements. Previously, Gratama [89] demonstrated, in our laboratory, that cardiovascular and hematological variables and organ blood flows were very stable on subsequent experimental days in lambs with and without an aorta-pulmonary shunt. In order to establish whether a similar stability was present in lambs with a right-to-left shunt, we compared cardiovascular and hematological variables and organ blood flows (Tables 8.1 and 8.2), obtained in two subsequent experiments. These results indicate that the hypoxemic lambs had a stable degree of hypoxemia and that their cardiovascular and hematological variables were similar on the two experimental

Table 8.1 Comparison of cardiovascular and hematological variables in hypoxemic lambs in two subsequent experiments.

	Experiment 1	Experiment 2
Age (d)	36±2	40±2*
Body mass (kg)	10.3±2.5	10.3±2.3
Oxygen saturation (%)		
arterial	64±8	67±8
mixed venous	39±10	40±11
coronary sinus	15±6	17±6
Hemoglobin concentration (g/L)	144±10	142±14
Hematocrit (%)	46±2	45±4
Blood pressure (mmHg)		
mean arterial	80±8	76±11
mean right atrial	7±5	7±6
Systemic blood flow (mL/min·kg)	181±70	200±78
Oxygen uptake (mL/min·kg)	8.8±2.3	8.9±2.2
Systemic oxygen supply (mL/min·kg)	23±10	26±13
Arterial pH, blood gases and bicarbonate		
pH	7.39±0.03	7.38±0.04
P_{CO_2} (kPa)	4.4±1.0	4.3±0.8
P_{O_2} (kPa)	7.3±1.7	7.9±1.4
bicarbonate (mmol/L)	19±4	18±4

Data obtained on the first experimental day (experiment 1) compared with data obtained on the subsequent experimental day (experiment 2) in 11 hypoxemic lambs. * $p<0.05$ by paired t-test

days. In addition, the organ blood flows were not significantly different on the two experimental days, but this could only be compared in 4 hypoxemic lambs.

Systemic blood flow (Qs) was calculated by using the Fick method {$Qs=VO_2/(CaO_2-CmvO_2)$}. We were concerned about bi-directional shunting of blood across the atrial septal defect, because this would lead to overestimation of systemic blood flow in hypoxemic lambs. However, there are several findings indicating that no significant atrial left-to-right shunt was present in the hypoxemic lambs. First, in 3 hypoxemic lambs studied by using the dye-dilution technique, no atrial left-to-right shunt was detected, whereas a large right-to-left shunt was clearly demonstrated (data not shown). Second, we compared systemic blood flow in the lambs in our study with those reported by other investigators, who used either electromagnetic flow transducers, radiolabeled microspheres, or the Fick method. Our results in both control and hypoxemic lambs are comparable to those obtained by others using any of the three techniques (Table 8.3). This is in accordance with the observation that each of these three techniques yields similar values of systemic blood flow in lambs [130]. Third, systemic blood flow, arterial oxygen concentration, and oxygen uptake are similar in hypoxemic and control lambs, whereas the mixed venous oxygen concentration is lower in hypoxemic lambs as compared with control lambs (Table 8.3), which argues against a significant atrial left-to-right shunt.

Alveolar hypoxic hypoxemia versus right-to-left shunt hypoxemia

Much information about the adjustments to chronic hypoxemia has been obtained from studies in humans and animals exposed to high altitude or simulated high altitude. In (simulated) high altitude hypoxemia the alveolar oxygen tension is

Table 8.2. Comparison of organ blood flow (mL/min·100g) in hypoxemic lambs in two subsequent experiments.

	Experiment 1	Experiment 2
Left ventricular free wall	172±63	178±48
Right ventricular free wall	190±87	210±83
Septum	193±89	209±77
Brain	109±59	87±22
Kidney	436±281	342±146
Thyroid	119±78	85±67
Adrenal	286±205	165±64
Gastrointestinal tract	108±62	81±24

Data obtained on the first experimental day (experiment 1) compared with data obtained on the subsequent experimental day (experiment 2) in 4 hypoxemic lambs. No significant differences by paired t-test.

decreased and all systemic venous return passes through the pulmonary circulation, so that effective pulmonary blood flow is normal. In contrast, in cardiac right-to-left shunt hypoxemia the alveolar oxygen tension is normal or somewhat increased, but a part of the systemic venous return bypasses the pulmonary circulation, so that the effective pulmonary blood flow is decreased. In this section we discuss differences and similarities between these forms of hypoxemia with respect to the cardiovascular, hematological, and growth adjustments. For more information about adjustments to high altitude the reader is referred to recently published reviews [8, 51, 155].

One of the most important differences between the two forms of hypoxemia is

Table 8.3 Comparison of systemic oxygen supply and its determinants, and oxygen uptake in hypoxemic and normoxemic lambs.

study	Dalinghaus[a]		Lister	Teitel		Bernstein	
method of Qs measurement	Fick		Fick	flow probe		microspheres	
defect	con	hyp	con	con	hyp	con	hyp
n	23	21	11	12	7	9	7
heart rate	140±29	163±30*	115±18	190	235*	180	220*
S_{aO_2}	93±2	67±6	90-95		61±6	94±2	75±2*
C_{aO_2}	133±17	124±28					
S_{mvO_2}	57±5	38±7			32±7	53±5	40±5*
C_{mvO_2}	82±13	70±25*					
$C_{(ao\text{-}mv)O_2}$	50±8	54±21	42±12				
Q_s	167±42	169±63	200±25	170±40	170±60	190±25	220±25
V_{O_2}	8.3±1.9	8.2±1.9	8-9		12±2	8±2	12±2
SOS	22±5	20±8	27±12		20±2	21±2	25±3

Comparison of systemic oxygen supply and its determinants, and oxygen uptake in normoxemic and in chronically hypoxemic lambs in three laboratories. Different methods for measuring systemic blood flow were used in each study. [a] Pooled data from control and hypoxemic lambs in our study, each lamb was only used once, compared with data from Lister et al. [138], Teitel et al. [213], and from Bernstein et al. [28]. S_{aO_2} = arterial oxygen saturation (%), C_{aO_2} = arterial oxygen concentration (mL/L), S_{mvO_2} = mixed venous oxygen saturation (%), C_{mvO_2} = mixed venous oxygen concentration (mL/L), $C_{(ao\text{-}mv)O_2}$ = arterio mixed venous oxygen concentration difference (mL/L), Q_s = systemic blood flow (mL/min·kg), V_{O_2} = oxygen uptake (mL/min·kg), SOS = systemic oxygen supply (mL/min·kg). * Hypoxemic lambs significantly different from normoxemic lambs as reported in the study.

the reaction of the pulmonary vascular bed. Alveolar hypoxia induces an increase of the pulmonary vascular resistance in humans and animals [9, 15, 47, 95, 141, 193, 194], although the magnitude of this increase varies between species. Chronic alveolar hypoxia increases pulmonary vascular resistance more than acute alveolar hypoxia [216] and induces structural changes in the pulmonary vasculature [176, 216]. The pulmonary resistance rapidly returns to normal after the termination of acute alveolar hypoxia [47, 141, 194], but not after the termination of chronic alveolar hypoxia [95, 176], suggesting that the structural changes in the pulmonary vascular bed are an important factor in the increased resistance. Although the mechanism that induces the increased pulmonary resistance is unknown, it is likely that the alveolar hypoxia per se is important. Thus, the structural changes in the pulmonary vasculature that accompany chronic alveolar hypoxia would not be expected in our hypoxemic lambs, because they had a normal alveolar oxygen tension. To what extent the increased whole blood viscosity contributes to the increased pulmonary resistance is uncertain; both a considerable contribution [12] and a negligible contribution have been claimed [216]. However, when the lung is perfused at a lower than normal flow rate, pulmonary resistance increases disproportionately with increasing hematocrit [165]. This indicates that in our experimental set-up the increased whole blood viscosity may have a considerable effect on pulmonary resistance. It is noteworthy that the pulmonary arterial pressure in our hypoxemic lambs was not decreased (Table 3.2), despite the fact that the pulmonary blood flow was approximately 50 % lower than in control lambs, indicating that pulmonary resistance was increased. However, we found no evidence of structural changes that caused an increased pulmonary resistance: after deflating the occluder around the pulmonary artery in our hypoxemic lambs, thereby acutely inducing normoxemia [105], the pulmonary arterial pressure was not increased, despite the increase in pulmonary blood flow. After a prolonged reduction of the pulmonary blood flow, for example in Tetralogy of Fallot, a restriction of the pulmonary vascular bed has been demonstrated [175], which may lead to an increased pulmonary resistance after correction of the lesion. It is probable that the duration of the decreased pulmonary blood flow in our lambs was too short to induce such changes in the pulmonary vasculature.

Right ventricular hypertrophy develops during chronic alveolar hypoxia as a consequence of the increased pulmonary resistance [15, 158, 176, 193, 216, 220]. Similarly, right ventricular hypertrophy developed in our hypoxemic lambs (Fig. 6.1), because of the pressure load imposed on the right ventricle by the occluder around the pulmonary artery. In contrast, systemic arterial pressures, atrial pressures, and systemic blood flow in chronically hypoxemic subjects are similar to those in normoxemic subjects, both in (simulated) high altitude and in cardiac

right-to-left shunt hypoxemia [95, 177, 213]. Thus, the cardiovascular "environment" in (simulated) high altitude and in cardiac right-to-left shunt hypoxemia is quite similar.

The hemoglobin concentration is increased in humans exposed to (simulated) high altitude [154]. Even infants born at high altitude have an increased hemoglobin concentration [7]. The hemoglobin concentration increases with higher altitude [154] and compensates for the arterial hypoxemia, because the arterial oxygen concentration is similar to that found in subjects at sea-level [20]. The role of a decreased oxygen affinity is uncertain: it has been demonstrated that the oxygen affinity decreases in humans at high altitude [134]. However, the decrease in standard P50 was offset by the effects of hyperventilation, so that the *in vivo* P50 in high altitude subjects was similar to that in sea-level subjects [144]. Many animals subjected to (simulated) high altitude increase their hemoglobin concentration in a fashion quite similar to that in humans. However, in some species that have been exposed to high altitude during evolution, the oxygen affinity is increased and the hemoglobin concentration is low at high altitude [33, 120, 206, 207]. Moreover, these animals seem to tolerate (simulated) high altitude hypoxemia better than animals with a "normal" oxygen affinity [33, 120]. Similarly, a better tolerance of high altitude hypoxemia has been demonstrated in man with a mutant high-affinity hemoglobin [104]. Thus, the hematological adaptation to (simulated) high altitude hypoxemia in humans and many animals is quite similar to that in cardiac right-to-left shunt hypoxemia. However, natural selection seems to favor an increased oxygen affinity and a consequent low hemoglobin concentration as adaptation to chronic alveolar hypoxia.

High altitude hypoxemia affects growth, both prenatally and postnatally. In humans the frequency of low birth weight increases with altitude and birth weight is inversely related to altitude [224, 241, 243]. In these studies unfavorable socioeconomic and nutritional factors were not clearly present. Instead, the decreased birth weight at high altitude has in part been related to the maternal arterial oxygen saturation during pregnancy [157], which in turn did depend on the increase of the hypoxic ventilatory response [156]. Postnatal growth in North American children at high altitude was decreased and this could only in part be explained by the lower birth weight [244]. Puberty starts later at high altitude, and is prolonged, although the final height and weight are not clearly decreased [77]. In young (experimental) animals subjected to simulated high altitude, a decreased growth rate is a common finding as well [15, 163, 221].

Oxygen capacity and oxygen affinity

One of the central themes of this thesis is how the oxygen supply to the tissues is maintained in chronic hypoxemia. We focused on the oxygen transport by the blood, although we recognize that ventilatory adjustments [134], especially in

alveolar hypoxia, as well as adjustments at tissue level that facilitate the diffusion of oxygen from the capillaries to the cells, may contribute considerably to the adjustments to chronic hypoxemia. The increase of the oxygen capacity of the blood, which facilitates oxygen supply to the tissues, is counterbalanced by the effect of the increased whole blood viscosity, which may impede oxygen supply to the tissues. In this section the contributions of the increased oxygen capacity, due to the increased hemoglobin concentration, and a decreased oxygen affinity are considered. The effect of the increased whole viscosity is considered in the next section.

Oxygen capacity

An increased hemoglobin concentration has long been recognized as an important adjustment to chronic hypoxemia [8, 134, 155, 171, 179, 185, 230], irrespective of the cause of hypoxemia. In congenital cyanotic heart disease the hemoglobin concentration is inversely related to the arterial oxygen saturation [26, 80, 82], while it also tends to increase with age [80]. Generally, the increase of the hemoglobin concentration is sufficient to maintain the arterial oxygen concentration at normoxemic levels [26]. After exposure to hypoxemia the increase of the hemoglobin concentration is brought about by an increased erythropoiesis, which is induced by an increased erythropoietin level [118]. In the early phase of exposure to hypoxemia, erythropoietin levels are increased [98, 197]. However, erythropoietin levels are in the normal range after prolonged hypoxemia [97, 197], indicating that an increased erythropoietin level is not necessary to sustain the increased hemoglobin concentration. The adjustments in chronically hypoxemic lambs are in accordance with these observations. Soon after the induction of hypoxemia the hemoglobin concentration starts to increase [213] and compensates for the decreased arterial oxygen saturation (Chapter 3). The twofold increase in oxygen capacity of the blood in hypoxemic lambs (Chapter 5) underscores the importance of the erythropoietic response in the adjustment to hypoxemia.

While it is true that the increased hemoglobin concentration compensates for the decreased arterial oxygen saturation, this compensation may not be sufficient in more severe forms of cyanotic heart disease. Relative anemia due to iron deficiency has been reported in children with cyanotic heart disease [81, 185, 235], and was found with comparatively lower arterial oxygen saturations [81, 185]. Similarly, an increased erythropoietin level has been demonstrated in a subgroup of cyanotic children that had lower arterial and mixed-venous oxygen saturations, lower oxygen tensions, increased oxygen extraction, and an increased red cell 2,3-DPG [82, 222]. These data suggest that in these conditions the compensation of the hypoxemia by the increased hemoglobin concentration is not sufficient. However, systemic blood flow was not increased in this subgroup of patients and

systemic oxygen supply was similar as compared with other cyanotic children, indicating that these variables cannot be used to distinguish this subgroup of patients. It is uncertain whether it should be attempted to increase the hemoglobin concentration in these conditions. Beneficial effects of an acute increase of the hemoglobin concentration have been demonstrated in mildly cyanotic patients with relative anemia [21]. However, supplementary iron given to children with severe cyanotic heart disease to treat their iron deficiency, induced the deleterious effects of hyperviscosity [185], indicating that the benefits of increasing the oxygen carrying capacity should continuously be weighed against the disadvantageous effects of increasing the whole blood viscosity.

In our study the mean arterial oxygen saturation of hypoxemic lambs was 65-70 %. When compared with the clinical studies cited above, our lambs may be classified as having moderate to severe cyanotic heart disease. One of the most obvious differences between our lambs and children with cyanotic heart disease is the lower hemoglobin concentration in lambs. This could be related to the lower oxygen affinity of the hemoglobin of lambs as compared with humans. Because all lambs received parenteral iron supplementation throughout the study, the low hemoglobin concentration in our lambs cannot be explained by iron deficiency.

Oxygen affinity

The exact role of a decreased oxygen affinity of hemoglobin as an adjustment to chronic hypoxemia is unclear. Theoretically, a decreased oxygen affinity is an advantageous adjustment when hypoxemia is caused by a right-to-left shunt [183]. The oxygen loading in the lungs is hardly affected because of the sigmoid shape of the oxygen dissociation curve, while the oxygen unloading to the tissues is facilitated. Consequently, the arterial and mixed-venous oxygen tension increase [183], which should increase the tissue oxygen tension [214]. Indeed, this effect of decreasing the oxygen affinity has been demonstrated in dogs with a right-to-left shunt [198].

To appreciate to what extent the adjustments in oxygen capacity and oxygen affinity in the hypoxemic lambs in our study can be extrapolated to congenital cyanotic heart disease in children, some important differences between the properties of hemoglobin in humans and in sheep must be discussed. The oxygen affinity of hemoglobin is determined by intrinsic properties of the hemoglobin, but modified by extrinsic factors. An increase in temperature, P_{CO_2}, H^+-concentration (decrease in pH), and an increase in 2,3-DPG all decrease the oxygen affinity. For the purpose of this discussion we shall only consider the differences in the intrinsic properties of hemoglobin and the effects of 2,3-DPG on oxygen affinity, because these factors are most important in the adjustment to chronic hypoxemia that is caused by a right-to-left shunt. In humans, fetal and adult hemoglobin have

intrinsically a similar affinity for oxygen [187]. Adult hemoglobin is stabilized in the deoxy-configuration by 2,3-DPG, in a 1:1 molar ratio, but in fetal hemoglobin this effect of 2,3-DPG is absent [187]. In sheep, fetal and three types of adult hemoglobin can be distinguished, each with intrinsically a different affinity for oxygen. There is no allosteric effect of 2,3-DPG on fetal or adult hemoglobin [18, 114, 143]. Thus, 2,3-DPG decreases the oxygen affinity of human adult hemoglobin, whereas it has no allosteric effect on human fetal hemoglobin or on ovine fetal and adult hemoglobin.

The oxygen affinity of fetal hemoglobin is higher than that of adult hemoglobin, so that the oxygen affinity changes in the newborn period. Immediately after birth the fraction of fetal hemoglobin is high both in humans and in sheep, but it gradually decreases as fetal hemoglobin is replaced by adult hemoglobin. Consequently, the oxygen affinity decreases in both species. In infants the decrease in oxygen affinity is related to the fact that increasing amounts of adult hemoglobin are available on which 2,3-DPG can act. By the age of 4-6 months the fraction of fetal hemoglobin is low in infants and the oxygen affinity reaches the adult level [52]. In lambs the decrease in oxygen affinity is related to the fact that high-affinity fetal hemoglobin is replaced with low-affinity adult hemoglobin. By the age of 5-6 weeks the fraction of fetal hemoglobin is low in lambs and the oxygen affinity is at the adult level [18, 138]. However, during the first ten days of life a high level of red-cell 2,3-DPG is found in lambs, which thereafter decreases to reach the low adult level by 20 days of life [10, 17]. The increased 2,3-DPG concentration causes a decrease in the intra-erythrocytic pH, which decreases the oxygen affinity through the Bohr-effect, so that the oxygen affinity in newborn lambs decreases much faster than can be explained by the replacement of fetal by adult hemoglobin alone [10, 16, 17]. Whether this effect of 2,3-DPG in newborn lambs is unique to neonatal red cells is unknown, but no reports are available that demonstrate similar effects in older lambs or adult sheep. Thus, the intrinsic oxygen affinity of hemoglobin and the effect of 2,3-DPG on hemoglobin is considerably different between humans and sheep.

Since the effect of 2,3-DPG on the affinity of adult hemoglobin has been disclosed [23, 38], the relation between a decreased oxygen affinity and increased red cell 2,3-DPG has been described in patients with chronic hypoxemia, anemia, and cardiac failure [61, 167, 219, 239]. In children with cyanotic heart disease a consistent decrease in oxygen affinity is not found before the age of 6 months, because of the presence of fetal hemoglobin [26, 231]. Thereafter, the increase in the red-cell 2,3-DPG and the P_{50} seem to be related to the severity of the hypoxemia, as is suggested by the inverse relationship between the P_{50} and arterial oxygen saturation or oxygen tension, mixed venous oxygen saturation, red cell pH, and erythropoietin levels [26, 82, 146, 231]. Consequently, in patients with mild hypoxemia

no decrease in oxygen affinity is found, suggesting that the increased oxygen capacity alone compensates for the decreased arterial oxygen saturation. We studied hematological and cardiovascular variables and oxygen affinity in children with congenital cyanotic and non-cyanotic heart disease during cardiac catheterization (Table 8.4). In these children the oxygen affinity in each group was not significantly different from that found in healthy adults. Moreover, there was no relation between the arterial or mixed venous oxygen saturation, oxygen tension, and the oxygen affinity, indicating that a decreased oxygen affinity is not required for a successful adjustment to chronic hypoxemia. We suggest, therefore, that oxygen affinity does not decrease in chronic hypoxemia unless the compensation by the increased hemoglobin concentration is not sufficient.

Because of the differences between human and sheep hemoglobin, the

Table 8.4 Cardiovascular and hematological data in children with congenital (cyanotic) heart disease during cardiac catheterization.

	Non-hypoxemic	Hypoxemic
Number	10	12
Age (yr)	10.2±5.7	4.4±4.6*
Body mass (kg)	37±17	14±9*
Hemoglobin (g/L)	134±15	157±17*
Hematocrit	41±4	44±5
Oxygen saturation (%)		
arterial	98±2	80±7*
mixed venous	73±6	60±9*
Systemic blood flow (mL/min·kg)	137±51	173±74
Systemic oxygen supply (mL/min·kg)	24±8	28±9
Oxygen uptake (mL/min·kg)	5.8±1.9	7.4±1.2
Oxygen extraction (%)	25±6	27±6
P50 (kPa)	3.63±0.14	3.66±0.14
2,3-DPG (mol/mol Hb)	0.89±0.16	0.98±0.19
pH (art)	7.38±0.03	7.41±0.04
PaO_2 (kPa)	12.1±1.6	4.4±0.5*
$PaCO_2$ (kPa)	5.2±0.7	4.4±0.5*

These data were obtained in children that underwent diagnostic cardiac catheterization in our laboratory. All measurements were obtained at rest. Non cyanotic subjects had an arterial oxygen saturation > 94%; diagnosis in this group included (supravalvular) aortic stenosis (n=2), tetralogy of Fallot (n=2), ASD (n=3) + mitral insufficiency (n=2), VSD (n=1), pulmonary hypertension (n=1), Fontan correction (n=1). Cyanotic children had an arterial oxygen saturation < 90%, diagnosis in this group included tricuspid atresia (n=2), tetralogy of Fallot (n=2), single ventricle, total A-V canal (n=1), VSD + persistent ductus Botalli (n=1), and complex cyanotic lesions (n=5). * Hypoxemic subjects significantly different from non-hypoxemic by unpaired t-test.

results in lambs with experimental cyanotic heart disease cannot simply be extrapolated to human congenital cyanotic heart disease. However, our results confirm the importance of an increased oxygen capacity as an adjustment to chronic hypoxemia. It normalizes the arterial oxygen concentration and also allows any volume of oxygen to be unloaded with a smaller fall in oxygen tension. This means that with increasing oxygen extraction in a capillary bed, the difference between the end-capillary oxygen tension of hypoxemic and normoxemic subjects will become smaller (Fig. 8.1). It is also clear from this figure that the initial fall in oxygen tension in normoxemic subjects is much greater than in hypoxemic subjects, because of the sigmoid shape of the oxygen dissociation curve.

Indirectly, the beneficial effect of a decreased oxygen affinity is confirmed in our study, because the hemoglobin concentration of hypoxemic lambs is much lower than would be expected for infants and children with a similar degree of hypoxemia, who have a higher normal hemoglobin concentration. Nevertheless, the disadvantageous effect of a moderately increased whole blood viscosity that is related to the increased hematocrit, can already be demonstrated in hypoxemic

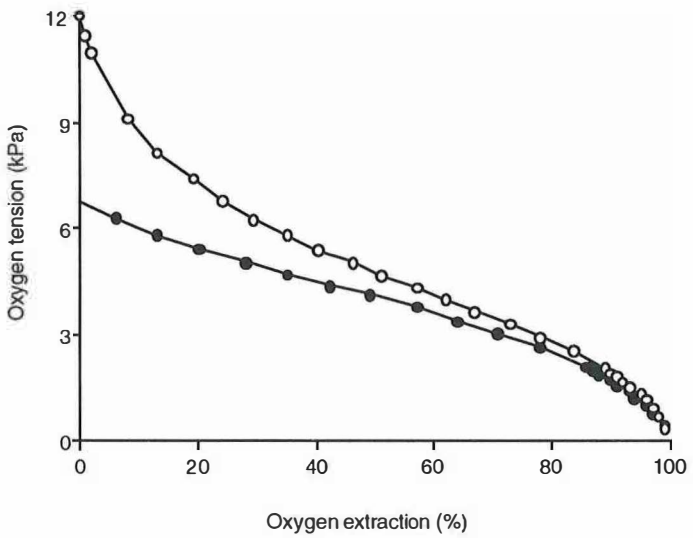

Figure 8.1 Oxygen tension of blood in control (open dots) and hypoxemic lambs (solid dots) as a function of oxygen extraction. At 0 % the arterial oxygen tension is displayed, at 100 % the theoretical situation when all oxygen is extracted. Oxygen tensions were calculated by using the ODC's of control and hypoxemic lambs (Chapter 3). Because the arterial oxygen concentration in hypoxemic lambs was similar to that in control lambs, hypoxemic and control lambs have unloaded a similar amount of oxygen at each level of oxygen extraction.

lambs (Chapter 4). Directly, the beneficial effect of a decreased oxygen affinity cannot be tested in our experimental set-up, because sheep do not have the ability to decrease their oxygen affinity any further. From a teleological point of view this is not surprising, because a further decrease of the oxygen affinity in sheep may impair the oxygen loading in the lungs under normal conditions (Fig. 8.2), which would offset the facilitated unloading of oxygen in the tissues. Because the position of the human oxygen dissociation curve is much more to the left (Fig. 8.2), a decrease of the oxygen affinity in humans will affect the oxygen loading in the lungs considerably less. Although an increase of the oxygen capacity is the primary adjustment to chronic hypoxemia, a decrease of the oxygen affinity in humans with cyanotic heart disease may prevent an excessive increase of the hemoglobin concentration and thus a further increase in whole blood viscosity.

Blood flow distribution and (myocardial) oxygen uptake

Not much information is available about the distribution of systemic blood flow in chronic hypoxemia. Clinical studies have evaluated the effects of severe polycythemia on systemic blood flow and oxygen supply, and the beneficial effects

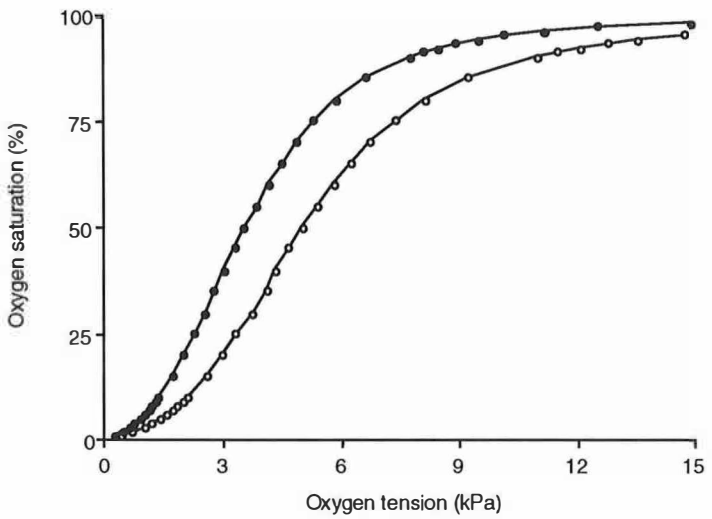

Figure 8.2 Oxygen dissociation curve of human (solid dots) and of lamb's (open dots) blood under standard conditions, ie for human blood t=37°C, pH=7.40 and $PaCO_2$=5.33kPa and for lambs t=39°C, pH=7.40 and $PaCO_2$=5.33kPa. The curve for humans is the mean ODC of 45 healthy adult subjects and was obtained by using the same technique as described in Chapter 3 (W.G. Zijlstra, personal communication). The curve for lambs is the mean ODC of all lambs described in Chapter 3. The P50 for the human curve is 3.55±0.18 kPa, for the lamb's curve it is 5.11±0.44 kPa.

of reducing the hematocrit in these conditions have been demonstrated [180, 181]. The effects of reducing the hematocrit on organ blood flow have indirectly been demonstrated by the disappearance of symptoms of hyperviscosity. Cerebral vascular accidents in young children with congenital cyanotic heart disease have been related to a decreased and sluggish cerebral blood flow, secondary to an increased whole blood viscosity [44]. In this regard, it has been demonstrated that the viscosity of microcytic blood is more increased than that of normocytic blood due to a decreased deformability of small red cells [136]. This observation has been used to explain why cyanotic children with relative anemia have an increased risk for cerebral vascular accidents, even at relatively low hematocrits [44]. One report described two young adults with cyanotic heart disease and severe polycythemia, suffering from myocardial infarction, presumably related to the sluggish coronary blood flow [242]. Although these studies define clinically important sequelae of (severe) polycythemia, they contribute little to our knowledge of blood flow distribution in chronic hypoxemia under normal conditions.

Systemic blood flow was not increased in hypoxemic lambs, but redistributed towards the heart and the brain and away from other organs (Chapter 4) [31] (Fig. 8.3). This redistribution of blood flow was related to vasodilation in the heart and the brain and to the effects of an increased whole blood viscosity (Chapter 4). Consequently, oxygen supply to most organs, except to the heart and the brain,

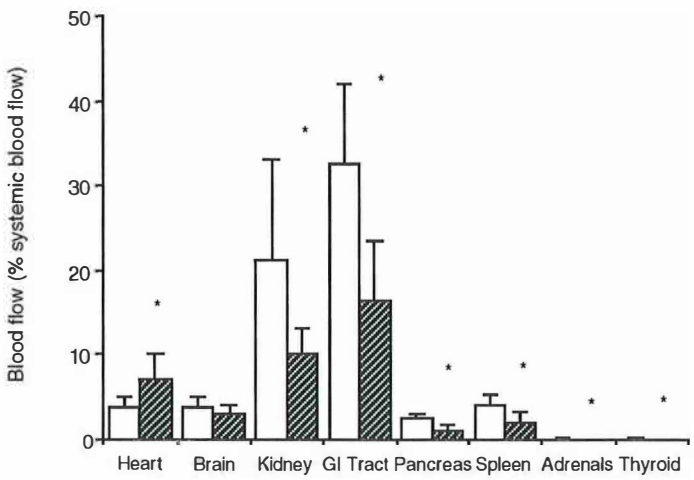

Figure 8.3 Organ blood flow in control (open bars) and hypoxemic lambs (hatched bars) as a percentage of systemic blood flow. Data derived from Chapter 4. * $p<0.05$ by unpaired Student's t-test.

decreased. Because systemic oxygen uptake was maintained in hypoxemic lambs, we assume that oxygen uptake by the organs was maintained as well, by using the oxygen extraction reserve. However, oxygen requirements for cardiorespiratory work will be increased in hypoxemic lambs. We estimate that the total oxygen uptake of the ventricular myocardium will be twofold increased as compared with control lambs and accounts for 12 % of systemic oxygen uptake as compared with 6 % in control lambs (Table 8.5). The redistribution of oxygen consumption towards the heart in hypoxemic lambs may be established at the expense of growth. It has been demonstrated that young lambs can decrease a part of their resting systemic oxygen uptake without developing signs of oxygen lack [107, 161, 201]. It has been suggested that the oxygen uptake for this so-called "non-essential" metabolism might be expended on growth. This may explain why the increased requirements for cardiorespiratory work in our hypoxemic lambs could be met without increasing the systemic oxygen uptake. During acute hypoxemia superimposed on chronic hypoxemia, blood flow to the heart and brain was further increased in order to maintain oxygen supply to these organs. In contrast, blood flow to other organs remained unaltered during acute hypoxemia, further decreasing the oxygen supply. Nevertheless, whole body oxygen uptake in our hypoxemic lambs was similar to that in control lambs and maintained during acute hypoxemia.

Some investigators have questioned the adequacy of myocardial oxygen delivery in chronically hypoxemic subjects. In dogs with experimental cyanotic heart disease, high energy phosphate stores were similar to those in normoxemic dogs [125, 204]. However, after 60 min of cardiopulmonary bypass high energy phosphate stores were decreased in cyanotic dogs, but not in normoxemic dogs [204]. In addition, an impairment of ventricular function has been demonstrated in dogs with experimental cyanotic heart disease [140]. During isoproterenol infusion

Table 8.5 Estimation of total myocardial oxygen uptake in control and hypoxemic lambs.

	Control lambs	Hypoxemic lambs
number	15	14
VO_2 (mL/min)	86±35	88±34
MVO_2 LV free wall (mL O_2/mL QLV free wall)	0.10±0.02	0.09±0.2
MVO_2 Total heart (mL O2/min)	5.1±2.2	10.0±3.7*
MVO_2/VO_2 (%)	6±2	12±5*

Estimated total oxygen uptake by the ventricular myocardium. Calculations based on data from Chapter 6. MVO_2 = myocardial oxygen uptake, Q = blood flow, VO_2 = whole body oxygen uptake. MVO_2 Total heart is calculated by assuming that the MVO_2 LV free wall per unit blood flow can be applied for all ventricular parts. Thus, MVO_2 Total heart = MVO_2 LV free wall x QTotal heart.

in dogs with acutely induced cyanotic heart disease, lactate was produced by the myocardium, whereas in non-cyanotic dogs no lactate was produced [87]. These results have been interpreted to indicate an inadequate oxygen delivery to the myocardium. In contrast, our results strongly suggest an adequate oxygen delivery to the myocardium in hypoxemic lambs (Chapter 6). Left ventricular oxygen uptake increased proportionately with oxygen demand and left ventricular oxygen supply was closely matched to oxygen uptake (Fig. 6.4). During acute hypoxemia superimposed on chronic hypoxemia, myocardial blood flow increased further in order to maintain oxygen supply. Moreover, during steady state hypoxemia or during superimposed acute hypoxemia we could not demonstrate signs of a metabolic derangement of myocardial metabolism.

Another question that remains unanswered is to what extent the decreased blood flow to the organs interferes with organ function. It has been demonstrated that the decreased blood flow to the gastrointestinal tract does not lead to gastrointestinal dysfunction [27]. However, to what extent the decreased blood flow to the small intestine interferes with substrate uptake from the gastrointestinal tract is uncertain. Also, renal blood flow and renal plasma flow were significantly decreased in hypoxemic lambs, whereas the GFR was maintained (Chapter 5). We estimated that the glomerular filtration fraction was twofold increased in hypoxemic lambs and speculated that the balance between glomerular afferent and efferent tone was altered in order to maintain GFR. Similarly, in patients with cyanotic heart disease the GFR is normal. However, in adults with cyanotic heart disease an increased plasma urate concentration has been reported and this was related to a decreased fractional urate excretion [142, 182], presumably secondary to the increased filtration fraction. In addition, glomerular enlargement has been demonstrated in children with cyanotic heart disease [229] and glomerular sclerosis has been described. Such alterations have been described when glomerular filtration pressure is increased for prolonged periods. A longstanding change in the balance between afferent and efferent glomerular arteriolar tone might explain how cyanotic heart disease in the long-term may lead to renal impairment.

Growth and congenital cyanotic heart disease

Growth retardation has been clearly documented in congenital heart disease [19, 67, 135, 188, 212]. The severity of the observed growth retardation depends in part on the method of patient selection. In large studies, growth retardation has been identified in certain subgroups of patients: children younger than two years [212], children with a large left-to-right shunt and signs of congestive heart failure [67, 212], and children with cyanotic heart disease [135, 188, 212]. When interpreting these data, one should be aware that the older studies included patients with congenital heart defects that were not yet amenable to early corrective surgery. The spectrum of growth

disturbances that was found in patients with congenital heart disease 20-30 years ago, has now dramatically changed [173]. Nevertheless, these studies still may provide useful information about the effects of congenital heart disease on growth.

Growth retardation in cyanotic heart disease seems to be related to the presence rather than to the degree of hypoxemia [135]. Both weight and height gain are decreased in cyanotic children in the first 2 years of life [188, 212]. In 8 children with tetralogy of Fallot weight and height age were lower than chronological age, while the ratio of weight and height age was relatively normal [83]. After corrective surgery, before the age of 2 years, these indices of growth did not change significantly, which was in sharp contrast to the observations made in children with large left-to-right shunts [83]. These results suggest that cyanotic heart disease induces a proportional growth retardation, with a limited ability for catch-up growth. In contrast, non-cyanotic heart disease with a large left-to-right shunt induces growth retardation with wasting, but a good potential for catch-up growth. If true, this may indicate a different mechanism of growth retardation in cyanotic as opposed to non-cyanotic heart disease.

Several mechanisms have been proposed to explain the growth retardation in congenital heart disease. These include a decreased energy intake, an increased energy expenditure, gastrointestinal dysfunction, hypoxia, and hormonal alterations. Energy intake per unit body mass in children with heart disease and growth failure was adequate [129, 188, 212] or in the low-normal range [100, 152]. However, it has been suggested that energy requirements should be based on recommended daily allowances for height [129, 225]. If such an approach is used, a decreased energy intake can be demonstrated for almost all children with heart disease and growth failure. Energy expenditure has been estimated by determining oxygen uptake per unit mass and was in the normal range for most patients, but it was increased in patients with congestive heart failure [152, 240]. It is noteworthy that forced feeding of children with congenital heart disease and growth failure, either orally by supplying high density caloric formula or by tube feeding, significantly increased energy intake and weight and length gain, without deteriorating the clinical condition [35, 129, 196, 225, 228, 240]. Irrespective of the exact mechanism of growth failure, an alteration of the balance between energy supply and demand restored growth rate to the normal range in these children, indicating that "caloric" treatment of growth failure in children with congenital heart disease is feasible. To what extent these results apply to all forms of cyanotic heart disease is uncertain. Although all of the cited studies included children with cyanotic heart defects and no difference in response was reported between cyanotic and non-cyanotic children, almost all children with growth failure had some degree of congestive heart failure as well. Thus, the growth failure and the response to the increased

energy intake may have been related to congestive heart failure rather than to hypoxemia.

Gastrointestinal dysfunction severe enough to explain growth failure in children with heart disease has not been demonstrated, not even during forced high energy intake [152, 208, 240]. In lambs with experimental cyanotic heart disease, intestinal lactase content and specific activity were approximately 50 % lower than in control lambs [27], but the importance of this finding to the growth failure is unclear.

Tissue hypoxia does not readily explain growth failure in cyanotic heart disease: clinical and experimental evidence, including the work presented in this thesis, does not support the contention that chronic hypoxemia leads to tissue hypoxia under normal conditions. However, hypoxemia may lead to a decrease of postnatal growth as demonstrated in patients with cyanotic heart disease [83], in animals with experimental cyanotic heart disease (Chapter 3) [29, 213], and in humans and animals exposed to (simulated) high altitude.

Alterations in growth hormone, somatomedins and insulin have been reported in congenital (cyanotic) heart disease. It has been proposed that growth hormone mainly stimulates cell proliferation through the effects of somatomedins, whereas insulin stimulates cytoplasmatic growth [39, 223]. Growth hormone concentration was increased in children with cyanotic heart disease as compared with non-cyanotic heart disease [64]. In contrast, growth hormone concentration was not significantly increased in lambs with experimental cyanotic heart disease as compared with control lambs, but insulin-like growth factor-I (IGF-I) was decreased [29]. This suggests that cell proliferation may be decreased in chronic hypoxemia, which may explain the difference in growth disturbance observed in young mice exposed to undernutrition and to chronic hypoxemia [163]. However, the interpretation of these results is complicated by the fact that the effects of IGF may vary between tissues and may vary with developmental stage [174]. Moreover, IGF binding proteins regulate the bio-availability of IGFs, so that the concentration of IGF alone does not determine its growth promoting effects. Insulin concentrations were increased after an oral glucose tolerance test in children with cyanotic heart disease [99]; but increased insulin concentrations would not explain growth retardation. Thyroid hormone concentrations are normal in congenital heart disease [22, 32] and do not explain the decreased growth rate. To elucidate the role of hormonal factors in the growth disturbance in chronic hypoxemia, further studies will be necessary.

How do the studies in lambs with experimental cyanotic heart disease by Teitel et al., Bernstein et al., and from our laboratory contribute to our knowledge of growth failure in cyanotic heart disease? The lambs in these studies have cyanotic heart disease with a decreased pulmonary blood flow and show no signs of congestive heart failure. The gain in body mass in these lambs is decreased after

the induction of hypoxemia and the lower body mass can be attributed to a decrease in the intracellular volume (Chapter 5).

Further analysis of our data demonstrates that myocardial mass and total red cell mass are increased and gastrointestinal mass is decreased in hypoxemic

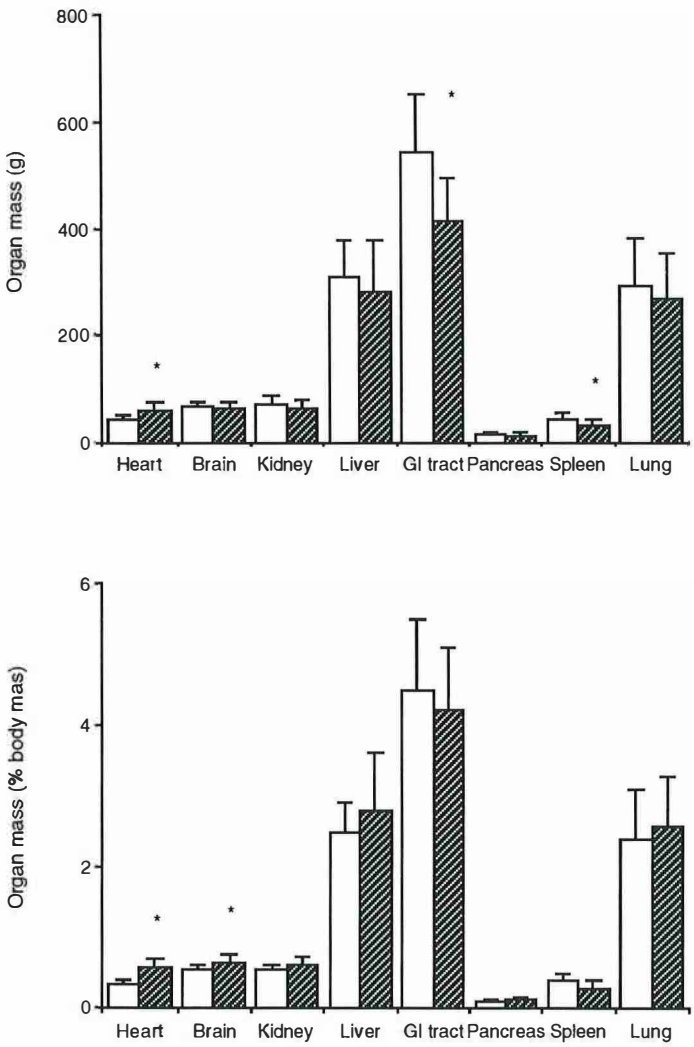

Figure 8.4 Organ mass in 18 control (open bars) and 20 hypoxemic lambs (hatched bars) in gram (upper panel) and as a percentage of body mass (lower panel). Data were obtained on the day of autopsy. GI tract = gastrointestinal tract, these data were obtained in 11 control and 12 hypoxemic lambs. * $p<0.05$ by unpaired Student's t-test.

lambs (Fig. 8.4). The mass of the brain, the heart, and the red cells per unit body mass are increased in hypoxemic lambs (Fig. 8.4), suggesting that brain growth is spared. No decreased energy intake in bottle-fed hypoxemic lambs [27], no increased oxygen uptake (Chapter 3) [213], and no gastrointestinal dysfunction are found in these lambs [27]. However, blood flow is redistributed away from the gastrointestinal tract, especially from the small intestine (Chapter 4) [31]. The alterations in growth hormone and IGF-I levels may either be the cause or a consequence of the growth failure [29]. Thus, cyanotic heart disease without congestive heart failure causes a decrease in the growth rate, but the mechanism of growth failure remains to be elucidated.

Conclusions

This study describes the cardiovascular, hematological and growth adjustments to experimental cyanotic heart disease in lambs. The increased oxygen capacity of the blood, through an increased red cell production, is the major adjustment to chronic hypoxemia and restores the arterial oxygen concentration to normoxemic levels. Conversely, adjustments of other components of the oxygen transport system contribute little to the adjustment to chronic hypoxemia. The increased ventilation hardly improves the oxygenation of hemoglobin in the lung; systemic blood flow is not increased, but systemic oxygen supply is maintained by the effects of the increased oxygen carrying capacity, and oxygen affinity is not decreased to facilitate the unloading of oxygen to the tissues. The blood flow to the organs is significantly affected by the effects of the increased whole blood viscosity. The blood flow and oxygen supply to the heart and the brain are maintained by vasodilation, whereas blood flow and oxygen supply to all other organs tend to be decreased. Nevertheless, our results do not support the contention that tissue hypoxia is present in resting conditions in chronic hypoxemia. Instead, we demonstrated that the adjustments to chronic hypoxemia result in a sufficient reserve in the oxygen transport system to cope with the additional stress of acute hypoxemia. In these conditions, oxygen supply to the heart and the brain is maintained, whereas oxygen supply to most other organs decreases. These responses are quite similar to those observed during acute derangements of oxygen transport in normoxemia.

Growth rate is decreased in lambs with experimental cyanotic heart disease and is related to a decreased intracellular volume. The decreased growth rate is related to hypoxemia and not to other aspects of the experimental set-up or to congestive heart failure. A part of the growth is expended directly on the adjustments to hypoxemia, such as increasing the myocardial and the red cell mass. The mechanism of growth failure in chronic hypoxemia remains uncertain,

but a decreased availability of oxygen for growth and a disturbance of the hormonal regulation of growth remain attractive subjects for further research.

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Summary

Congenital cyanotic heart disease is the most important cause of chronic hypoxemia in infancy and childhood. Because early corrective surgery is not feasible in the majority of cases, most of the affected infants have to adjust to hypoxemia. Although studies in these infants and children have contributed considerably to our understanding of the pathophysiology and the sequelae of chronic hypoxemia, many questions still remain unresolved.

In this thesis our studies on adjustments to chronic hypoxemia in lambs with experimental cyanotic heart disease are described. We focused on four main issues. First, we determined to what extent the various components of the oxygen transport system contributed to maintaining the oxygen requirements of the tissues. Therefore, the determinants of systemic oxygen supply and oxygen uptake were studied in chronically hypoxemic lambs. In addition, the effect of the increased whole blood viscosity, that develops as a consequence of the increased hemoglobin concentration, on organ blood flow and oxygen supply was studied. Second, the oxygen supply to and oxygen uptake by the left ventricular myocardium were studied in relation to left ventricular oxygen demand. In addition, left ventricular substrate uptake was studied. Third, the effects of chronic hypoxemia on growth were studied, by determining the development of body mass in hypoxemic lambs, and by assessing whether other factors in our experimental design contributed to the decreased growth rate. Furthermore, body fluid compartment volumes were studied in order to determine the effects of chronic hypoxemia on lean body mass and its components. Fourth, acute hypoxemia superimposed on chronic hypoxemia was studied by acutely increasing the cardiac right-to-left shunt to determine whether the ability to respond to an acute further decrease in the arterial oxygen saturation was blunted after 3-4 weeks of hypoxemia.

Chronic hypoxemia in the lambs was induced by the combination of an atrial septal defect and a variable pulmonary stenosis. The lambs underwent surgery before the 10th day of life, when an atrial septal defect was created by means of a balloon septostomy, an inflatable constrictor was placed around the main pulmonary artery, and intravascular catheters were inserted. After 3-5 days of recovery from surgery, the constrictor around the main pulmonary artery was inflated, in order to induce a cardiac right-to-left shunt, and to lower the arterial oxygen saturation to 60-70 %. After 3-4 weeks of hypoxemia measurements were made. In control lambs only intravascular catheters were inserted during surgery, or neck vessel catheters were inserted under local anesthesia.

The contribution of cardiovascular and hematological adjustments, in maintaining the systemic oxygen supply and oxygen uptake in chronically hypoxemic lambs, are described in Chapter 3. Specifically, the contribution of the

oxygen capacity of blood and the oxygen affinity of hemoglobin were determined. The arterial oxygen saturation was 66±8 % in hypoxemic lambs, but the increase of the oxygen capacity of blood (hemoglobin concentration) was sufficient to maintain the arterial oxygen concentration at a similar level as in control lambs. Because systemic blood was maintained in hypoxemic lambs, at the expense of an increased heart rate, systemic oxygen supply was maintained as well. Systemic oxygen uptake in hypoxemic lambs was similar to that in control lambs. The oxygen affinity was low in lambs and did not decrease any further in chronic hypoxemia; the erythrocyte 2,3-DPG concentration was low. Growth rate was 30 % lower in hypoxemic as compared with control lambs, and the decreased growth rate was related to chronic hypoxemia and not to other factors in the experimental design. These results demonstrate the importance of the increased oxygen capacity of blood as an adjustment to chronic hypoxemia, whereas a decrease in the oxygen affinity does not seem to be essential for a successful adjustment. In addition, these results indicate that the adjustment to chronic hypoxemia is established at the expense of a normal growth rate.

The effect of the increased whole blood viscosity, that develops as a consequence of the increased hemoglobin concentration, on organ blood flow and oxygen supply is described in Chapter 4. Whole blood viscosity was increased by approximately 25 % in chronically hypoxemic lambs. The blood flows to the gastrointestinal tract, the liver, the kidneys and the thyroids were decreased in hypoxemic lambs as compared with control lambs, whereas the blood flows to the heart and the brain were maintained. The decrease in blood flow to organs was related to the increased whole viscosity, because the vascular tone in these organs of the hypoxemic lambs was similar to that of the control lambs. In contrast, blood flows to the heart and brain were maintained by a compensatory decrease in vascular tone in these organs. Because the arterial oxygen concentration in hypoxemic lambs was similar to that in control lambs, the oxygen supply to the heart and brain was maintained, whereas it was decreased to most other organs. Because the systemic oxygen uptake in hypoxemic lambs was similar to that in control lambs, we assume that the decreased oxygen supply to organs was compensated by an increased oxygen extraction in the organs, in order to meet the oxygen requirements of the tissues. To what extent the decreased blood flow to organs interferes with other organ functions remains to be determined. These results indicate that the beneficial effect of the increased oxygen capacity of blood, as an adjustment to chronic hypoxemia, is in part offset by the negative effect of the increased whole blood viscosity on organ blood flow.

The effect of chronic hypoxemia on body fluid compartment volumes is described in Chapter 5. Intracellular volume was decreased in hypoxemic lambs, whereas their extracellular volume was similar to that in control lambs. The lower

intracellular volume in hypoxemic lambs could almost completely account for the decrease in body mass. Per unit body mass, total body water was similar in hypoxemic and control lambs, but extracellular volume was increased and intracellular volume was decreased in hypoxemic lambs. Consequently, the ratio of extracellular to intracellular volume was increased. In addition, blood volume was increased in hypoxemic lambs, mainly by an increase in total red cell volume. The total amount of hemoglobin was twofold increased in hypoxemic lambs, which underscores the importance of the hematopoietic response in the adjustment to chronic hypoxemia. These results suggest that the growth retardation in hypoxemic lambs is related to a decreased cellular growth. However, no conclusions can be drawn about the mechanism of the decreased growth of the cellular compartment, i.e. whether it is related to a decreased rate of cell division or to a decreased cell volume.

The effects of chronic hypoxemia on left ventricular oxygen supply and oxygen uptake, and on substrate supply and uptake are described in Chapter 6. Myocardial mass was increased in hypoxemic lambs, mainly because of the increased right ventricular mass. Similarly, myocardial blood flow was twofold increased, mainly because of the increased right ventricular blood flow. Presumably, this was related to the increased work load imposed on the right ventricle by the pulmonary stenosis. Blood flow to the left ventricle was increased, in order to increase its oxygen supply, and to meet the increased oxygen demand of the left ventricle. Left ventricular oxygen uptake was increased in hypoxemic lambs, which could be explained by the increased heart rate, because per beat the oxygen uptake was similar in hypoxemic and control lambs. Left ventricular oxygen uptake increased with oxygen demand. Similarly, left ventricular oxygen supply increased linearly with increasing oxygen uptake. There were slight differences in the arterial substrate concentrations between hypoxemic and control lambs, but we could not detect any differences in left ventricular substrate uptake between hypoxemic and control lambs. Free fatty acids and β-hydroxybutyrate were the most important substrates taken up by the left ventricle, that we identified. However, the total substrate uptake that we measured could only supply 50 % of the energy requirements of the left ventricle. We suspect that triglycerides and short chain fatty acids may also be important substrates for the myocardium in these lambs.

The cardiovascular responses to acute hypoxemia superimposed on chronic hypoxemia are described in Chapter 7. This experiment was carried out to establish whether the chemoreceptor-mediated and adrenergic responses to acute hypoxemia were blunted after 3-4 weeks of chronic hypoxemia. Acute hypoxemia was induced by further inflating the constrictor around the pulmonary artery, thereby acutely increasing the cardiac right-to-left shunt. The arterial oxygen saturation decreased from 65 % to 45 % and consequently the arterial oxygen concentration

decreased by 37 %. In response, systemic blood flow increased by 41 %, maintaining systemic oxygen supply at the same level as before the induction of acute hypoxemia. Blood flow to the heart and the adrenals increased and blood flow to the brain also tended to increase, so that the oxygen supply to these organs was maintained. In contrast, blood flow to most other organs was unaltered, so that the oxygen supply to these organs decreased. Systemic and myocardial oxygen uptake were maintained and no metabolic acidosis developed during acute hypoxemia. These results suggest that systemic and myocardial oxygen requirements were met during acute hypoxemia. The responses to acute hypoxemia superimposed on chronic hypoxemia were quite similar to those that have previously been described for acute hypoxemia in normoxemic lambs. Therefore, we conclude that after 3-4 weeks of hypoxemia the response to superimposed acute hypoxemia is not blunted in lambs.

In Chapter 8 the results of our studies are discussed in relation to the results of other studies on chronic hypoxemia, either induced by (simulated) high altitude or by (experimentally induced) congenital cyanotic heart disease. We conclude that the adjustments to chronic hypoxemia in lambs in our study are quite similar to those described in other studies. The cardiovascular and hematological adjustments to chronic hypoxemia in lambs are qualitatively comparable to those in other mammalian species, including man. However, the low oxygen affinity in lambs and the fact that their oxygen affinity cannot decrease any further, makes direct comparison with human data impossible. Nevertheless, our study underscores the importance of an increased oxygen capacity as an adjustment to chronic hypoxemia. The disadvantage of a moderate increase of the whole blood viscosity on organ blood flows is clearly demonstrated, although a compensatory decrease in the vascular tone in the heart and the brain maintains blood flow and oxygen supply to these organs. We demonstrated that the oxygen requirements of the left ventricular myocardium are met during chronic hypoxemia, as well as during acute hypoxemia superimposed on chronic hypoxemia. Moreover, the cardiovascular response to acute hypoxemia in chronically hypoxemic lambs was adequate to maintain oxygen supply to the tissues. Finally, this study confirms the growth retardation in chronic hypoxemia and expands previous observations by demonstrating how body fluid compartment volumes are affected by chronic hypoxemia. In conclusion, the adjustments to chronic hypoxemia in lambs with experimental cyanotic heart are qualitatively similar to those described in other mammalian species. In this way our study contributes to a further insight into the pathophysiology of the adjustments to chronic hypoxemia.

Samenvatting

Bij 15-20 % van de aangeboren hartafwijkingen is de zuurstofverzadiging van het arteriële bloed verlaagd (hypoxemie). Deze hartafwijkingen zijn de belangrijkste oorzaak van chronische hypoxemie op de kinderleeftijd. Ze komen bij 1-2 per 1000 levendgeborenen voor. In het merendeel van de gevallen is correctie ervan kort na de geboorte niet goed mogelijk, zodat aanpassing aan de hypoxemie zal plaatsvinden. Bij patiënten is veel onderzoek gedaan naar die aanpassingen en de gevolgen daarvan. Dit onderzoek heeft veel bijgedragen aan onze kennis van dergelijke hartafwijkingen, maar door de heterogeniteit van de onderzochte groepen patiënten èn door de beperkte mogelijkheden om invasieve metingen te verrichten bij patiënten, blijven nog veel vragen onopgelost. Op een deel van deze vragen wordt getracht een antwoord te geven in dit proefschrift, waarin het onderzoek wordt beschreven naar aanpassingen aan chronische hypoxemie bij lammeren, waarbij zo'n hartafwijking was nagebootst.

Onder normale omstandigheden stroomt zuurstofarm bloed uit het lichaam naar de rechter boezem (atrium) van het hart en wordt vervolgens door de rechter kamer (ventrikel) naar de longen gepompt. Hier wordt zuurstof gebonden aan het transporteiwit (hemoglobine), dat het bloed zijn rode kleur geeft. Tijdens de passage door de longen wordt het hemoglobine vrijwel volledig met zuurstof verzadigd. Vanuit de longen stroomt zuurstofrijk bloed naar het linker atrium, waarna het door de linker ventrikel naar het lichaam wordt gepompt. In de weefsels geeft het hemoglobine de zuurstof gedeeltelijk af, waarna het zuurstofarme bloed terugstroomt naar het hart. Hiermee is de cirkel gesloten. Bij een deel van de aangeboren hartafwijkingen bestaat er een abnormale verbinding tussen de bloedsomloop van de longen en die van het lichaam, waarbij een deel van het zuurstofarme bloed rechtstreeks naar de bloedsomloop van het lichaam stroomt. Een dergelijke verbinding wordt een rechts-links shunt genoemd en heeft een verlaagde zuurstofverzadiging van het arteriële bloed tot gevolg.

Opzet van het onderzoek

Bij de lammeren in dit onderzoek werd zo'n hartafwijking met een rechts-links shunt nagebootst, door een verbinding tussen het rechter en het linker atrium (atriumseptumdefect) te maken in combinatie met een vernauwing van de longslagader (pulmonaalstenose). Door de pulmonaalstenose was er een belemmering voor zuurstofarm bloed uit het lichaam om naar de bloedsomloop van de longen te stromen, waardoor een deel van dit bloed via het atriumseptumdefect naar het linker atrium stroomde, zodat een rechts-links shunt ontstond (Fig. 2.1). De lammeren werden in de eerste of tweede levensweek geopereerd, waarbij het atriumseptumdefect werd gemaakt en een opblaasbaar bandje om de hoofdstam van de longslagader werd aangebracht, dat van buiten het lichaam kon worden

opgeblazen. Tevens werden catheters op verschillende plaatsen in het hart en de bloedvaten gebracht, zodat later gemakkelijk bloed afgenomen kon worden en bloeddrukken konden worden gemeten. Enkele dagen na de operatie werd het bandje rond de longslagader opgeblazen, waardoor hypoxemie ontstond. De metingen werden verricht nadat de hypoxemie 3-4 weken had bestaan. De resultaten werden vergeleken met die van normoxemische lammeren, die als controlegroep dienden.

Aanpassingen in het zuurstoftransport

Het zuurstofaanbod aan het lichaam wordt bepaald door de lichaamsdoorstroming, de zuurstofcapaciteit van het bloed - die weer bepaald wordt door de hemoglobineconcentratie - en de arteriële zuurstofverzadiging. Bij (chronische) hypoxemie kan het zuurstofaanbod aan het lichaam op peil worden gehouden door een toename van de lichaamsdoorstroming, door een toename van de hemoglobineconcentratie, of door een combinatie van deze twee factoren. Een toename van de lichaamsdoorstroming speelt een belangrijke rol bij acute hypoxemie, maar na enige dagen keert de lichaamsdoorstroming terug naar de uitgangswaarde. Een toename van de hemoglobineconcentratie is een trager verlopende aanpassing, die een belangrijke rol speelt bij chronische hypoxemie. Over het algemeen compenseert deze toename de hypoxemie, zodat de zuurstofconcentratie van het bloed niet verschillend is van de waarde, die bij een normale arteriële zuurstofverzadiging wordt gevonden.

Bij de hypoxemische lammeren in dit onderzoek was de arteriële zuurstofverzadiging aanzienlijk verlaagd (65-70 %), maar de toegenomen hemoglobineconcentratie compenseerde de hypoxemie volledig, zodat de zuurstofconcentratie van het arteriële bloed bij hypoxemische en normoxemische lammeren gelijk was. De lichaamsdoorstroming was niet toegenomen bij de hypoxemische lammeren, hoewel de hartfrequentie verhoogd was. Het zuurstofaanbod aan het lichaam was dus hetzelfde bij hypoxemische en normoxemische lammeren (hoofdstuk 3). De totale hoeveelheid hemoglobine was verdubbeld bij hypoxemische lammeren (hoofdstuk 5), hetgeen het belang van de toegenomen zuurstofcapaciteit nog eens onderstreept. De zuurstofopname van het lichaam was bij de hypoxemische lammeren even hoog als bij de normoxemische lammeren.

De zuurstofaffiniteit van het hemoglobine is een tweede factor van belang bij het zuurstoftransport naar de lichaamscellen. Voor een effectief transport moet hemoglobine niet alleen in de longen zuurstof binden, maar deze in de weefsels ook weer afgeven. Dit proces is afhankelijk van de zuurstofspanning en wordt beschreven door de zuurstofdissociatiecurve (Fig. 8.2). Bij een verlaging van de zuurstofaffiniteit kan het hemoglobine de zuurstof gemakkelijker afgeven, maar ook

moeilijker binden ("de zuurstofdissociatiecurve schuift naar rechts"). Aangezien de zuurstofspanning in de longen relatief hoog is (14-15 kPa), heeft een matige verlaging van de zuurstofaffiniteit betrekkelijk weinig invloed op de binding van zuurstof aan hemoglobine in de longen, maar deze verlaging heeft wel een aanzienlijk effect op de zuurstofafgifte in de weefsels. Hoe belangrijk een daling van de zuurstofaffiniteit is voor een succesvolle aanpassing aan chronische hypoxemie is echter onduidelijk.

Bij de hypoxemische lammeren was de zuurstofaffiniteit van het hemoglobine niet verlaagd (hoofdstuk 3). Het lijkt dat het hemoglobine van schapen de mogelijkheid heeft verloren om de zuurstofaffiniteit verder te verlagen. Hoewel een verminderde zuurstofaffiniteit geen rol speelt bij het lam, blijkt de aanpassing aan chronische hypoxemie op soortgelijke wijze te verlopen als bij de mens en bij diersoorten waarbij de zuurstofaffiniteit wel kan veranderen. Hieruit kan geconcludeerd worden dat een verandering van de zuurstofaffiniteit niet cruciaal is voor een succesvolle aanpassing aan chronische hypoxemie. Dit onderzoek toont op indirecte wijze wel het voordeel van een lage zuurstofaffiniteit aan, doordat de hemoglobineconcentratie van het hypoxemische lam veel lager bleek te zijn dan die van een kind met een zelfde mate van hypoxemie.

Effect van de toegenomen viscositeit van het bloed

De hogere hemoglobineconcentratie komt tot stand door een toename van het aantal rode bloedcellen per liter. Hierdoor neemt de viscositeit (stroperigheid) en daarmee de stromingsweerstand van het bloed toe. Nadelige gevolgen van de hogere viscositeit van het bloed voor de lichaams- en orgaandoorstroming bij patiënten met chronische hypoxemie zijn eigenlijk alleen beschreven bij extreem hoge hemoglobineconcentraties. Het is echter niet goed bekend of er bij een matig toegenomen viscositeit, zoals meestal het geval is bij chronische hypoxemie, een effect op de doorstroming van organen is en in welke mate daarvoor gecompenseerd wordt.

In dit onderzoek werd de orgaandoorstroming gemeten met behulp van kleine, radioactief gemerkte bolletjes (microsferen), die in de kleinste bloedvaatjes van de organen blijven steken. De radioactiviteit in een orgaan was zo een maat voor de grootte van de doorstroming. Hoewel de viscositeit van het bloed bij hypoxemische lammeren maar matig verhoogd was, bleek de doorstroming van vrijwel alle organen, behalve die van het hart en de hersenen, verminderd te zijn (hoofdstuk 4). Bovendien bleek dat deze verminderde doorstroming het directe gevolg was van de toegenomen viscositeit van het bloed. De doorstroming van het hart en de hersenen werd daarentegen op peil gehouden, dankzij een compensatoire vaatverwijding. De zuurstofconcentratie van het bloed van hypoxemische lammeren was normaal, zodat het zuurstofaanbod aan de meeste

organen, behalve aan het hart en de hersenen, was verlaagd. Hieruit blijkt dat het gunstige effect van de toegenomen hemoglobineconcentratie op het zuurstoftransport gedeeltelijk teniet wordt gedaan door het gelijktijdig optredende, nadelige, effect van de toegenomen viscositeit van het bloed. Bovendien kan een verminderde orgaandoorstroming een negatief effect hebben op de aan- en afvoer van voedingsstoffen en afvalprodukten.

Stofwisseling van de hartspier

De zuurstofvoorziening van de hartspier lijkt goed gewaarborgd te zijn bij chronische hypoxemie, maar sommige onderzoekers hebben het tegendeel beweerd. De zuurstofbehoefte van de hartspier is hoog en dient continu gewaarborgd te zijn om (onbeschadigd) te kunnen overleven. Onder normale omstandigheden neemt de hartspier 70-80 % van de aangeboden zuurstof op (zuurstofextractie), zodat de mogelijkheid om deze extractie te vergroten vrij beperkt is. Daarom is het zuurstofaanbod aan de hartspier nauw gekoppeld aan de zuurstofbehoefte. Een vermindering van het zuurstofaanbod aan de hartspier of een toename van de zuurstofbehoefte van de hartspier wordt opgevangen door een toename van de doorstroming. De mate waarin aan de zuurstofbehoefte van de hartspier wordt voldaan bij chronische hypoxemie en de rol die de doorstroming daarin speelt, is niet goed bekend. Evenmin is bekend of de opname van voedingsstoffen (substraat) door de hartspier verandert onder invloed van chronische hypoxemie.

Bij hypoxemische lammeren was de doorstroming van de hartspier tweemaal zo hoog als bij normoxemische lammeren, voornamelijk door de toegenomen doorstroming van de rechter ventrikel (hoofdstuk 6). De zuurstofbehoefte van de spierwand van de linker ventrikel was licht verhoogd bij hypoxemische lammeren door de toegenomen hartfrequentie. Aan deze hogere zuurstofbehoefte werd voldaan door een evenredige toename van de doorstroming, waardoor het zuurstofaanbod aan de linker ventrikel toenam. De zuurstofbehoefte van de rechter ventrikel was waarschijnlijk sterk toegenomen door de hogere arbeid die de rechter ventrikel moest verrichten om het bloed door de pulmonaalstenose te pompen. Dit leidde tot een aanzienlijke hypertrofie, die onder andere blijkt uit de toegenomen massa van de rechter ventrikel (Fig 6.1). We hebben berekend dat de totale zuurstofbehoefte van de hartspier bij hypoxemische lammeren ongeveer 12 % van de zuurstofopname van het lichaam bedraagt, terwijl dit bij normoxemische lammeren slechts 6 % is. Uit dit onderzoek blijkt dat aan de toegenomen zuurstofbehoefte van de linker ventrikel wordt voldaan, terwijl daarover voor de rechter ventrikel geen zekere uitspraak gedaan kan worden. De verschillen in de zuurstofopname van de hartspier gingen niet gepaard met een verandering in de substraatopname.

Acute hypoxemie bij chronische hypoxemie

Bij hartafwijkingen die gepaard gaan met een rechts-links shunt, kunnen episodes voorkomen waarbij de arteriële zuurstofverzadiging plotseling daalt. De oorzaak van deze "hypoxic spells" is niet bekend, maar ze zijn zeer bedreigend voor de patiënt. Tijdens acute hypoxemie treden reacties op die tot doel hebben het zuurstofaanbod aan het lichaam, met name aan die organen die essentieel zijn voor overleving, op peil te houden. Nu is bekend dat na langdurige blootstelling aan hypoxemie de gevoeligheid van de sensoren, die bij deze reacties een belangrijke rol spelen, afneemt. Men kan zich dus voorstellen dat de aanpassing aan acute hypoxemie bij chronische hypoxemie minder goed verloopt dan bij normoxemie.

De aanpassing aan acute hypoxemie bij chronische hypoxemie werd bestudeerd door de rechts-links shunt acuut te laten toenemen, door het verder opblazen van het bandje om de longslagader. Hierdoor daalde de arteriële zuurstofverzadiging van 65 naar 45 %. De lichaamsdoorstroming en de doorstroming van hart en hersenen namen zodanig toe tijdens de acute hypoxemie, dat het zuurstofaanbod aan het lichaam en aan deze organen op peil werd gehouden. De doorstroming van de meeste andere organen bleef onveranderd, zodat het zuurstofaanbod aan deze organen daalde. De zuurstofopname van het lichaam en die van de hartspier bleven echter op peil. Evenmin ontstond er verzuring van het bloed (metabole acidose), zodat aangenomen mag worden dat aan de zuurstofbehoefte van het lichaam en van de hartspier werd voldaan. De aanpassingen aan acute hypoxemie bij chronisch hypoxemische lammeren zijn zowel kwalitatief als kwantitatief vrijwel gelijk aan de aanpassingen aan acute hypoxemie die eerder zijn beschreven bij normoxemische lammeren. Hieruit kan geconcludeerd worden dat na 3-4 weken hypoxemie de aanpassing aan acute hypoxemie ongestoord is.

Groei

Groeivertraging is een veel voorkomend probleem bij chronische ziekten op de kinderleeftijd, zo ook bij aangeboren hartafwijkingen. Het mechanisme van de groeivertraging is echter onduidelijk en waarschijnlijk spelen verscheidene factoren een rol. Zo kan het basale energieverbruik toegenomen zijn, de energie-opname of de hoeveelheid zuurstof die nodig is om de groei te garanderen kan verminderd zijn, of er kunnen hormonale veranderingen zijn die de groei negatief beïnvloeden. Bovendien is het mogelijk dat het mechanisme van de groeivertraging bij hartafwijkingen met hypoxemie anders is dan bij afwijkingen zonder hypoxemie.

De hypoxemische lammeren in onze studie hadden een 30 % lagere gewichtstoename dan de normoxemische lammeren. Onze resultaten tonen aan dat de groeivertraging gerelateerd is aan de hypoxemie en niet aan de chirurgische ingreep of het verblijf in het laboratorium (hoofdstuk 3). Eveneens blijkt uit onze resultaten dat het gewichtsverschil tussen hypoxemische en normoxemische lammeren vrijwel volledig verklaard wordt door een verschil in het intracellulaire volume (hoofdstuk 5). Een vertraagde cellulaire groei, door een verminderd celvolume of door een verminderd aantal cellen, is de meest waarschijnlijke verklaring voor de groei-achterstand van hypoxemische lammeren. Om een uitspraak te kunnen doen over het mechanisme van de vertraagde groei is echter verder onderzoek noodzakelijk.

Conclusie

Bij lammeren die chronisch hypoxemisch zijn door een experimenteel nagebootste hartafwijking, is de toegenomen hemoglobineconcentratie de belangrijkste aanpassing. Hierdoor wordt de zuurstofconcentratie van het arteriële bloed genormaliseerd, maar het gunstige effect op het zuurstoftransport wordt gedeeltelijk tenietgedaan door het negatieve effect van de toegenomen viscositeit van het bloed op de orgaandoorstroming. Desondanks wordt het zuurstofaanbod aan het lichaam en aan de voor overleving noodzakelijke organen op peil gehouden, zelfs tijdens een acute verdere daling van de arteriële zuurstofverzadiging. De groeivertraging die optreedt bij hypoxemische lammeren, kan toegeschreven worden aan een lager intracellulair volume, waarschijnlijk als gevolg van een vertraagde cellulaire groei. De aanpassingen aan chronische hypoxemie bij de lammeren in deze experimenten zijn goed vergelijkbaar met die bij mensen en andere diersoorten. Dit maakt een zinvolle vergelijking mogelijk van de resultaten van dit werk met die van andere in de literatuur. Op deze wijze draagt dit onderzoek bij aan een beter inzicht in de pathofysiologie van de aanpassingen aan chronische hypoxemie.