Restoration of cardiopulmonary function with 21% versus
100% oxygen after hypoxaemia in newborn pigs

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Objective: To assess the consequences of hypoxia and resuscitation with room air versus 100% O\textsubscript{2} on cardiac troponin I (cTnI), cardiac output (CO), and pulmonary artery pressure (PAP) in newborn pigs.

Design: Twenty anaesthetised pigs (12–36 hours; 1.7–2.7 kg) were subjected to hypoxia by ventilation with 8% O\textsubscript{2}. When mean arterial blood pressure fell to 15 mm Hg, or arterial base excess was \leq -20 mmol/l, resuscitation was performed with 21% (n = 10) or 100% (n = 10) O\textsubscript{2} for 30 minutes, then ventilation with 21% O\textsubscript{2} for 120 minutes. Blood was analysed for cTnI. Ultrasound examinations of CO and PAP (estimated from tricuspid regurgitation velocity (TR-Vmax)) were performed at baseline, during hypoxia, and at the start of and during reoxygenation.

Results: cTnI increased from baseline to the end point (p < 0.001), confirming a serious myocardial injury, with no differences between the 21% and 100% O\textsubscript{2} groups (p = 0.12). TR-Vmax increased during the insult and returned towards baseline values during reoxygenation, with no differences between the groups (p = 0.11) or between cTnI concentrations (p = 0.31). An inverse relation was found between increasing age and TR-Vmax during hypoxia (p = 0.034). CO per kg body weight increased during the early phase of hypoxia (p < 0.001), then decreased. Changes in CO per kg were mainly due to changes in heart rate, with no differences between the groups during reoxygenation (p = 0.298).

Conclusion: Hypoxia affects the myocardium and PAP. During this limited period of observation, reoxygenation with 100% O\textsubscript{2} showed no benefits compared with 21% O\textsubscript{2} in normalising myocardial function and PAP. The important issue may be resuscitation and reoxygenation without hyperoxygenation.

Myocardial dysfunction, hypotension, and increased pulmonary vascular resistance are well known consequences of hypoxic-ischaemic insults in neonates, with a relation between low alveolar oxygen tension and increased pulmonary vascular resistance. In contrast with chronic hypoxia, the effects are reversible with reoxygenation after insults of short duration.

Cardiac troponin I (cTnI) is a regulatory contractile protein. The troponin complex has three subunits in the thin filament of the muscle myofibrils, where cTnI is the actomyosin ATPase inhibiting subunit. It has been shown to be a specific and sensitive marker for myocardial damage in both human infants and an experimental model of newborn asphyxiated pigs.

In modern neonatal intensive care units, sick neonates are monitored with continuous electrocardiography, invasive blood pressure, respiratory rate, and saturation of oxygen in arterial blood. Doppler echocardiography is a useful non-invasive diagnostic method of assessing ventricular filling, ventricular function, and, more specifically, ventricular output and pulmonary artery pressure (PAP). Echocardiography may be useful in monitoring newborn animals as well.

Birth asphyxia is a worldwide problem leading to high morbidity and mortality. Treatment strategies in neonatal resuscitation are mainly based on tradition and, to a lesser extent, on evidence based medicine. The aim of successful resuscitation is to prevent both neonatal death and adverse long-term neurodevelopmental outcome associated with birth asphyxia. Oxygen delivery to organs must be sufficient to provide adequate oxygen consumption and prevent tissue acidosis. It is also important to prevent further tissue damage caused by reoxygenation and oxygen toxicity injury.

There is an ongoing debate on the optimal concentration of oxygen for neonatal resuscitation. International Guidelines for Neonatal Resuscitation in 2000 recommended resuscitation of the newborn infant with 100% oxygen. Several animal and also human studies have suggested that room air is as effective as, or may be even better than, 100% oxygen in resuscitation. The aim of this study was to assess the consequences of hypoxia and reoxygenation with 21% and 100% oxygen on cardiac output (CO) and PAP by Doppler echocardiography and on serum cTnI in a newborn pig model.

METHODS

Approval

The Norwegian Animal Experimental Board approved the experimental protocol. The animals were cared for and handled in accordance with the European guidelines for use of experimental animals.

Animal preparation

Of 28 anaesthetised and tracheotomised Landrace pigs, 20 (12–36 hours old, weight 1.7–2.7 kg) were included in the study. Three pigs were used as sham animals and treated as the experimental animals except for the hypoxic insult and reoxygenation. Five pigs had to be excluded, two because of diarrhoea/sepsis, and three died from complications from the hypoxic-ischaemic insult, such as serious bradycardia.

General anaesthesia was induced with halothane and discontinued when venous access was achieved, usually within two minutes. After induction, a bolus dose of 10 mg/kg ketobarbital, 10–30 μg/kg fentanyl, and 1 mg/kg midazolam was given. When tracheotomy had been performed, anaesthesia was given as a continuous infusion of 50 mg/kg/h fentanyl and 0.25 mg/kg/h midazolam.

Abbreviations: CO, cardiac output; cTnI, cardiac troponin I; LVP, left ventricular pressure; PAP, pulmonary artery pressure; TR-Vmax, peak tricuspid regurgitation velocity
Mechanical ventilation was performed with a pressure controlled ventilator (Babylog 8000+; Drägerwerk, Lübeck, Germany). Normoventilation (Paco₂ 4.5–6.0 kPa) with tidal volume 10–15 ml/kg was achieved by adjusting peak inspiratory pressure or ventilatory rate. The inspiratory time of 0.4 second and positive end expiratory pressure of 0.4 cm H₂O were kept constant throughout the experiment. Inspired fraction of O₂ and end tidal CO₂ were continuously monitored (CO₂SMO; Novametrix, Wallingford, Connecticut, USA).

Rectal temperature was maintained between 38 and 40°C with a heating blanket and a radiant heating lamp (normal pig temperature 38.5–39.5°C).

Arterial and venous catheters were inserted into the right femoral artery and left external jugular vein (polyethylene catheters Portex PE-50; Portex Ltd, Hythe, Kent, UK) for arterial blood pressure monitoring, blood sampling, and fluid infusions. Continuous intravenous infusion with Salidex (35 mg/ml glucose, 50 mmol/ml NaCl) was given, and blood glucose was kept in the range 2–10 mmol/l.

Guided by ultrasound, a 2F micro manometer-tipped catheter (MPC-500, Millar catheter; Millar Instruments, Houston, Texas, USA) was inserted from the left carotid artery into the left ventricle for continuous monitoring of left ventricular pressure (LVP). The electrocardiogram was continuously monitored.

**Experimental protocol**

The animals were stabilised for one hour after surgery. Hypoxaemia was achieved by ventilation with a gas mixture of 8% O₂ in N₂ (AGA, Oslo, Norway) until either mean arterial blood pressure reached 15 mm Hg or base excess was ≤−20 mmol/l. Before resuscitation, the pigs were block randomised into two groups. Resuscitation was performed with either 21% or 100% O₂ for 30 minutes. Then the pigs were ventilated with room air for another 120 minutes. At the end of the experiment, the pigs were given an overdose of 150 mg/kg pentobarbital intravenously.⁵

**Blood samples**

Blood gases corrected for the pig’s temperature were analysed after surgery, at baseline (H0), regularly through hypoxaemia, at the start and 10, 20, 30, and 120 minutes of reoxyenation, and at the end of the experiment. Haemoglobin was measured at baseline. Blood sampling for analysis of cTnI was collected at baseline and at the end of the study, centrifuged, and kept at −70°C until analysis with an AIO Immunoanalyzer (Turku, Finland).

**Ultrasound examinations**

A Vivid 7 ultrasound scanner (GE VingMed Ultrasound, Horten, Norway) with an integrated program for quantitative analysis for humans and combined tissue imaging, and Doppler transducer (10S) with a range of frequencies from 4.0 to 10.0 MHz, was used. The analyses were mainly performed with the factory default of 8.9 MHz.

Two investigators (DF, KL), one handling the probe and one dealing with the ultrasound equipment, performed the ultrasound examinations. The data were sampled when both agreed that the imaging quality was optimal. The recordings were stored on black and white paper prints as well as CD-ROM and MO discs for further analysis.

The ultrasonographic studies were performed after anaesthesia and surgical instrumentation, at baseline (H0), 30 minutes after the start of hypoxia (H30), at the start of reoxyenation (R0), and at 30, 90, and 150 minutes of reoxyenation (R30, R90, and R150). With the pigs lying in a right lateral, half supine position, the first examination was performed to exclude congenital heart defects and persistent ductus arteriosus, to measure the aortic valve diameter, and to guide the insertion of the Millar catheter. The diameter of the aortic valve was measured as a leaflet separation from a parasternal long axis view from the inner edge of the hinge points of the aortic valve. This diameter was used for all calculations of CO to limit calculation errors due to measurement inaccuracy.

Further investigations were performed with pigs lying in a left lateral, half supine position. The peak tricuspid

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### Table 1 Baseline values

<table>
<thead>
<tr>
<th></th>
<th>Sham (n = 3)</th>
<th>21% (n = 10)</th>
<th>100% (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (hours)</td>
<td>33.7 (2.2)</td>
<td>26.7 (8.2)</td>
<td>28.0 (8.7)</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>2.33 (0.45)</td>
<td>2.07 (0.29)</td>
<td>2.05 (0.37)</td>
</tr>
<tr>
<td>Haemoglobin (g/l)</td>
<td>6.8 (1.4)</td>
<td>6.9 (0.3)</td>
<td>6.9 (0.4)</td>
</tr>
<tr>
<td>Calcium (mm/l)</td>
<td>1.44 (0.04)</td>
<td>1.36 (0.02)</td>
<td>1.34 (0.12)</td>
</tr>
<tr>
<td>pH</td>
<td>7.47 (0.03)</td>
<td>7.49 (0.02)</td>
<td>7.44 (0.02)</td>
</tr>
<tr>
<td>Base excess (mmol/l)</td>
<td>5.94 (1.2)</td>
<td>5.28 (1.3)</td>
<td>2.54 (0.9)</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>132 (9)</td>
<td>152 (9)</td>
<td>165 (12)</td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td>66 (5)</td>
<td>68 (5)</td>
<td>66 (5)</td>
</tr>
<tr>
<td>Diastolic BP (mm Hg)</td>
<td>51 (5)</td>
<td>53 (4)</td>
<td>53 (5)</td>
</tr>
</tbody>
</table>

Age and body weight are mean (SD); all other values are mean (SEM). Hypoxaemia was induced in all the experimental animals. They were then randomised to one of two groups and resuscitated with either 21% or 100% O₂.

### Table 2 Values at end of insult (RO)

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>21%</th>
<th>100%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypoxaemia time (min)</td>
<td>7.48 (0.02)</td>
<td>7.08 (0.01)</td>
<td>7.10 (0.02)</td>
</tr>
<tr>
<td>pH</td>
<td>3.83 (1.76)</td>
<td>−21.07 (0.57)</td>
<td>−20.70 (0.70)</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>128 (8)</td>
<td>174 (21)</td>
<td>172 (18)</td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td>66 (4)</td>
<td>37 (6)</td>
<td>26 (3)</td>
</tr>
<tr>
<td>Diastolic BP (mm Hg)</td>
<td>49 (7)</td>
<td>31 (6)</td>
<td>21 (2)</td>
</tr>
</tbody>
</table>

Values are mean (SD). Hypoxaemia was induced in all the experimental animals. They were then randomised to one of two groups and resuscitated with either 21% or 100% O₂.

BP, Blood pressure.
regurgitation velocity (TR-Vmax, m/s) was sampled from the apical “four chamber view” to calculate the pressure gradient between the right ventricle and the right atrium (mm Hg) by using the inbuilt program in the ultrasound equipment applying the modified Bernoulli’s equation. CO (litres/min) was calculated from stroke volume (velocity time integral across the aortic valve x valve area) x heart rate from the apical “five chamber view”. Angle corrections were not used. All measurements used in the final calculation for each pig were the mean values, automatically calculated in the ultrasound scanner, from three to five consecutive beats regarded as good quality measurements.\(^{20}\)

### Statistical analysis

Statistical analysis was performed by SPSS11. Animal weight and age are given as mean (SD), and all other data are given as mean (SEM). Because of skewed distribution of cTnI, these variables were log transformed before analysis. For comparisons within two groups, the independent \(t\) test, non-parametric test, or repeated measurements was used as appropriate. To study the linear relation between two variables, linear regression analysis was used. Area under curve was used to obtain a summary measure of the variables. \(p<0.05\) was considered significant.

### RESULTS

There were no differences between the two groups with regard to numbers of animals, sex, body weight, age, or cardiovascular and biochemical variables at baseline (table 1). The duration of the insult, mean arterial blood pressure, heart rate, pH, and base excess at the end of the insult were similar in the two groups (table 2).

No pigs had any cardiac malformation, but four had patent ductus arteriosus. Two of them had an open duct from the first examination, whereas two reopened during the insult. All ducal shuntings were left-right, but the maximum systolic peak velocities were not measured because the angle errors were too large.

cTnI increased from baseline to end point (0.05 (0.003) \(\mu\)g/l; \(p<0.001\)) but with no differences between the 21% and 100% \(O_2\) group (\(p = 0.12\)). The analytical sensitivity of cTnI is 0.01 \(\mu\)g/l, but the laboratory performing the analysis gave the lowest values as <0.05 \(\mu\)g/l. Of 23 pigs, 20 had values lower than 0.05 \(\mu\)g/l. The sham piglets showed no change in cTnI (<0.5 \(\mu\)g/l).

Table 3 presents measured and calculated cardiac variables for the pigs. TR-Vmax increased in all pigs during the insult.
n = 10; 100% O\textsubscript{2}, n = 10). Values are mean (SEM) (sham, n = 3; 21% O\textsubscript{2}, reoxygenation (R0), and at 30, 90, and 150 minutes of reoxygenation (R30, R90, and R150). Values are mean (SEM) (sham, n = 3; 21% O\textsubscript{2}, n = 10; 100% O\textsubscript{2}, n = 10).

(2.38 (0.193) m/s at baseline v 2.91 (0.130) m/s at the start of resuscitation) and returned towards baseline values during reoxygenation, but with no differences between the groups (p = 0.11) (fig 1) or between cTnI concentrations (p = 0.310). An inverse relation was found between TR-Vmax and increasing age of the pigs during hypoxaemia (p = 0.034).

CO per kg increased during the early phase of hypoxaemia (0.31 (0.02) litres/min/kg at H0 v 0.42 (0.02) litres/min/kg at H30; p<0.001), and then decreased slightly (NS) (fig 2). Before resuscitation (R0) there was a difference between the two groups, with a higher mean CO per kg in the group resuscitated with 100% O\textsubscript{2}. One explanation for this is that pig no 10 in the group receiving room air developed pericardial effusion, severe mitral and aortic valve insufficiency, increasing flow across the ductus arteriosus, and severe tachycardia during the insult, with the heart rate up to 248 beats/min at H30. Then suddenly both heart rate and stroke volume decreased at R0 with a subsequent fall in CO per kg. Further, of the pigs randomised to 100% O\textsubscript{2}, pig no 23 had much higher CO per kg because of a high stroke volume (3.83 litres/min/kg) at R0 than any of the other pigs in both groups, and pig no 26 developed arteriovenous block and bradycardia during hypoxaemia, with subsequent severe tachycardia during reoxygenation. During reoxygenation, however, there were no group differences in CO per kg (p = 0.298). The overall changes in CO per kg were due mainly to changes in heart rate (fig 3) and not stroke volume (fig 4).

Adequate recordings of LVP of sufficient quality for further analysis were achieved in only eight pigs. The main problem was that the catheter easily became stuck in the ventricular wall or papillary muscles. LVP decreased during hypoxaemia and returned to normal values during reoxygenation, with no significant differences between the two groups. It changed in the same direction as CO per kg and inversely with TR-Vmax, but this was not significant using regression analysis or area under the curve, except at R150 where we found a relation between LVP max and TR-Vmax (p = 0.032).

Electrocardiograms were used to monitor arrhythmias and for Doppler analysis.

DISCUSSION

cTnI was used as a marker of myocardial injury to assess the difference between baseline and recovery. The increase in cTnI confirmed a severe myocardial injury. As shown previously, there was no protection in the 100% O\textsubscript{2} group during this short period of observation with regard to the amount of cTnI releases.\textsuperscript{5}

The pulmonary circulation constricts in response to acute hypoxia, which is reversible on re-exposure to O\textsubscript{2}.\textsuperscript{20} Hypoxic pulmonary vasoconstriction is the physiological state in fetal life. Increased O\textsubscript{2} at birth is a powerful stimulus inducing vasorelaxation.\textsuperscript{21} The reactivity of the pulmonary vessels to vasoreactive stimuli decreases with increasing age.\textsuperscript{22} The pulmonary vessels are especially vulnerable to hypoxic events stimulating vasoconstriction in the first days of life.\textsuperscript{21} Accordingly the youngest animals developed the highest PAP in this study.

The main treatment for persistent pulmonary hypertension of the newborn was for many years to correct acidosis, hypoxaemia, and hypercarbia with alkalinisation, hyperoxegenation, and induction of hypocarbia. However, hypocarbia has never been shown to reduce mortality or morbidity for this condition.\textsuperscript{21} Hyperventilation with 100% O\textsubscript{2} may actually worsen pulmonary and neurological morbidity.\textsuperscript{19} The stimulus-response relation between graded hypoxaemia and blood flow diversion in the lungs with regard to the hyperoxic part is open to debate. Hyperoxia has been reported to cause pulmonary vasodilatation,\textsuperscript{22} to have no effect on pulmonary vascular resistance\textsuperscript{2,22} but also to cause vasoconstriction.\textsuperscript{21,22} In a pig model, hypoxaemia induced pulmonary hypertension and plasma endothelin 1 were as effectively normalised when reoxygenation was performed with room air as with 100% O\textsubscript{2}.\textsuperscript{23} Our study supports this.

Despite a fall in blood pressure, we found an increase in heart rate and CO per kg in the early phase of hypoxaemia. We can only speculate on the reason for the short period of increased CO per kg while the systemic blood pressure fell. Neonates regulate their CO mainly by regulating heart rate,\textsuperscript{11} which is consistent with our results. Changes in blood pressure may reflect changes in CO and/or systemic vascular resistance. A fall in blood pressure will reduce left ventricular afterload and may for a short while improve myocardial performance. CO may be a more sensitive reflection of the haemodynamic situation than blood pressure alone.\textsuperscript{22} Left ventricular output has been suggested to be one of the haemodynamic features most closely linked to outcome in pulmonary hypertension of the newborn.\textsuperscript{21}

The subsequent fall observed in CO per kg during hypoxaemia is probably caused by increased acidosis and worsening of the cardiac pump function. Low pH and
accumulation of lactate may affect the interaction between calcium and troponin in the contractile mechanisms. An increased PAP will reduce left ventricular preload and lower reoxygenation was the same in both groups, as well as the cTn release from the myocardium, showing that 100% O₂ had no protective effect on the myocardium. LVP changed in the same direction as CO per kg and inversely with TR-Vmax. Increased PAP will decrease preload to the left ventricle, affect coronary perfusion, and, together with hypoxemia and acidosis, contribute to increasing left ventricular failure. Three pigs died from bradycardia. Hypoxemia stimulates the carotid body and effects reflex responses, involving hyperventilation, peripheral vasoconstriction mediated by a adrenergic sympathetic neurones, and vagally induced bradycardia. This may lead to profound bradycardia and sinusoidal heart rhythm. Hypoxia compromises the energetic state of the myocardium and disturbs the ionic balance. Ca²⁺ and reactive oxygen species play central roles in ischaemia-reperfusion damage possibly by affecting mitochondrial functions, resulting in myocardial cell injury and arrhythmias.

Study limitations
The pigs were heavily anaesthetised and “instrumented”. Even though we allowed a period of recovery and care was taken to minimise myocardial damage, baseline values were probably impaired compared with healthy “non-instrumented” conscious pigs. Doppler echocardiography as a method has several limitations. The sharp angle of the pig’s sternum makes it difficult to obtain exact parasternal long axis views. A small inaccuracy in measuring the diameter of the aortic valve will give rise to big differences in the calculated stroke volume. Angle defaults greater than 0–20° will also have a large impact on the results. In measuring CO, we actually measure velocity time integrals rather than using volumetric assessments directly. In clinical settings, there is little else to use other than the assessment of CO derived from Doppler measurements.

CONCLUSION
This study shows that a hypoxic-ischaemic insult affected the myocardium, the conducting system, and the PAP. The youngest animals responded with highest PAP. The pigs were heavily anaesthetised and "instrumented". Even though we allowed a period of recovery and care was taken to minimise myocardial damage, baseline values were probably impaired compared with healthy "non-instrumented" conscious pigs. Doppler echocardiography as a method has several limitations. The sharp angle of the pig’s sternum makes it difficult to obtain exact parasternal long axis views. A small inaccuracy in measuring the diameter of the aortic valve will give rise to big differences in the calculated stroke volume. Angle defaults greater than 0–20° will also have a large impact on the results. In measuring CO, we actually measure velocity time integrals rather than using volumetric assessments directly. In clinical settings, there is little else to use other than the assessment of CO derived from Doppler measurements.

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A baby was born with proptosis of the right eye at 35 weeks. The baby had required rotation by Keillands forceps, but delivery failed and the baby was delivered by emergency caesarean section after disimpaction of the head from the pelvis. At birth the baby was noted to have a complete proptosis of the right globe and a Keillands forceps mark over the right temporoparietal area but no direct orbital injury (fig 1). Both upper and lower eyelids were retracted exposing the sclera. The pupil of the proptosed eye was mid-sized and unreactive to light. The eye was immediately treated with lubricants and covered with geloperm to prevent exposure keratopathy and corneal ulceration. Spontaneous resolution of eye lid retraction occurred around one hour after birth leading to an appreciable reduction of the proptosis.

Non-contrast computed tomography of the head at 5 hours of age showed mild right eye proptosis with normal optic nerves and retro-orbital space. Oedema settled over five days, and on discharge only pupillary responses remained abnormal. At 4 weeks of age ophthalmic examination was entirely normal.

The likely differential diagnoses in cases such as this are: craniosynostosis; traumatic orbital congestion or retrobulbar bleed from instrumental delivery; or a retro-orbital mass. However, in the absence of direct orbital injury, we believe that the proptosis in this case was caused by a vacuum effect upon disimpaction of the head. Immediate computed tomography or magnetic resonance imaging after birth is essential to determine the diagnosis and treatment required. Simple protective measures and analgesia should be instituted before scanning is carried out.

Parental consent was obtained for publication of figure 1.

References