ORIGINAL ARTICLE

Resuscitation with 100% O_2 does not protect the myocardium in hypoxic newborn piglets

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Background: Perinatal asphyxia is associated with cardiac dysfunction secondary to myocardial ischaemia. Cardiac troponin I (cTnI) is a marker of myocardial necrosis. Raised concentrations in the blood are related to perinatal asphyxia and increased morbidity.

Objective: To assess porcine myocardial damage from enzyme release during hypoxaemia induced global ischaemia, and subsequent resuscitation with ambient air or 100% O_2. To investigate whether CO_2 level during resuscitation influences myocardial damage.

Design: Newborn piglets (12–36 hours) were exposed to hypoxaemia by ventilation with 8% O_2 in nitrogen. When mean arterial blood pressure had fallen to 15 mm Hg, or base excess to < -20 mmol/l, the animals were randomly resuscitated by ventilation with either 21% O_2 (group A, n = 29) or 100% O_2 (group B, n = 29) for 30 minutes. Afterwards they were observed in ambient air for another 150 minutes. During resuscitation, the two groups were further divided into three subgroups with different CO_2 levels.

Analysis: Blood samples were analysed for cTnI, myoglobin, and creatine kinase-myocardial band (CK-MB) at baseline and at the end of the study.

Results: cTnI increased more than 10-fold (p < 0.001) in all the groups. Myoglobin and CK-MB doubled in concentration.

Conclusion: The considerable increase in cTnI indicates seriously affected myocardium. Reoxygenation with 100% oxygen offered no biochemical benefit over ambient air. CK-MB and myoglobin were not reliable markers of myocardial damage. Normoventilation tended to produce better myocardial outcome than hyperventilation or hypoventilation.

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Birth asphyxia is still a major clinical problem, leading to high morbidity and mortality.[1] Annually, about 4 million neonates are affected world wide; approximately one million will die, and an equal number will develop serious sequelae.[2][3] Resuscitation after birth is a common procedure, and it is important to develop optimal resuscitation methods to prevent further injury. A major question is whether resuscitation should be performed with ambient air or 100% O_2.[4–6] Over the last few years, there has been growing evidence that ambient air is as efficient as 100% O_2.[4][5][7][8] After a period of hypoxaemia, sudden reintroduction of high concentrations of O_2 to hypoxic tissues may result in a burst of oxygen free radical formation, which may increase hypoxic tissue damage.[9][10] In the Resair 2 trial, it was found that neonates resuscitated with ambient air gave their first cry earlier than babies resuscitated with 100% O_2,[7][11] they achieved a sustained pattern of spontaneous respiration more rapidly,[11] and there were no differences in morbidity or mortality.[7][11] However, little attention has been paid to another important question, whether the ventilation frequency or modus operandi of resuscitation influences the outcome of the baby. Cardiac abnormalities in asphyxiated neonates are often underdiagnosed and require a high index of suspicion.[12][13] They may present with hypotension, which is associated with increased mortality and morbidity. The clinical picture is variable, and the severity of the diagnosis may range from tachypnoea to congestive heart failure and cardiogenic shock.[12–14]

Cardiac troponin I (cTnI) is a regulatory contractile protein. The troponin complex exists as a group of three subunits in the thin filament of muscle myofibrils. cTnI is the actomyosin ATPase-inhibiting subunit, and, when detected in the circulation, cTnI has been shown to be a sensitive and specific marker of myocardial damage in both the adult and paediatric population.[15][16] cTnI is unaffected by gestational age, birth weight, sex, and mode of delivery.[15][17] Neonates have higher cTnI concentrations than adults.[18] The aim of this study was to investigate cardiac involvement in a pig model that reproduces an asphyxial situation during labour by exposing newborn piglets to hypoxaemia and global ischaemia, and subsequent resuscitation with ambient air or 100% O_2. Myocardial damage was assessed by monitoring enzyme release. We also investigated if myocardial damage is influenced by the CO_2 level or ventilatory mode during resuscitation.

METHODS

Approval

The experimental protocol was approved by the hospital's ethics committee for animal studies under surveillance of the Norwegian Animal Experimental Board. The animals were cared for and handled in accordance with the European Guidelines for Use of Experimental Animals.

Surgical preparation

Seventy five newborn Landrace piglets (12 to 36 hours) were delivered from a local farmer (one hour transportation) on the day of the experiment. Seventeen were excluded, mainly because of low haemoglobin concentration at baseline, infection, metabolic acidosis, and diarrhoea. One was excluded because it died from hypoxaemia, one because of skull fracture, and three because of technical problems.

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Abbreviations: cTnI, cardiac troponin I; PAP, pulmonary artery pressure; PIP, peak inspiratory pressure; PVR, pulmonary vascular resistance

General anaesthesia was induced with 4% halothane (Fluothane; AstraZeneca, Södertälje, Sweden), reduced to 1–1.5% mixed with room air/100% O_2 until the piglet was unconscious. An ear vein was cannulated, and pentobarbital 50 mg/ml (Haukeland University Hospital Pharmacy, Bergen, Norway) 15–20 mg/kg, fentanyl (Leptanal; Janssen Pharmaceutica, Beerse, Belgium) 10–30 µg/kg, and midazolam (Dormicum; Hoffmann La Roche, Basel, Switzerland) 0.4 mg/kg was given. Lidocaine (Xylocain; AstraZeneca) 1% was given as local anaesthetic before tracheotomy. General anaesthesia was continued throughout the experiment, given as a continuous infusion with fentanyl 50 µg/kg and midazolam 0.25 mg/kg (IVAC P2000 infusion pump). If necessary, a bolus of fentanyl (10 µg) or midazolam (1 mg) was added. Before cannulation of the femoral artery, a bolus of pancuronium (Pavulon; NV Organon Oss, the Netherlands) 0.1 mg/kg was given to prevent shivering. A continuous intravenous infusion (saline 0.7% and glucose 1.25%) 20 ml/kg was given throughout the experiment. Glucose was regularly measured in the blood (Blood Gas Analyzer 860; Ciba Corning Diagnostics, Midfield, Massachusetts, USA), and intravenous infusion was occasionally adjusted to keep serum glucose in the selected range (2–10 mmol/l). Haemoglobin 50–110 g/l was confirmed after surgery (Co-oximeter 270; Instrumentation Laboratory, Lexington, Massachusetts, USA). Base excess and electrolytes were not adjusted.

After tracheotomy, a 3.5 mm Portex endotracheal tube (Portex Ltd, Hythe, Kent, UK) was inserted, with a ligature around the tube and trachea to prevent leakage and displacement of the tube. The piglets were ventilated mechanically with a pressure controlled ventilator, (Babylog 8000+; Drägerwerk, Lubeck, Germany). Normoventilation (Pa_{CO_2} 4.5–6.0 kPa) and a tidal volume 10–15 ml/kg was achieved by adjusting the peak inspiratory pressure (PIP) or ventilatory rate. During surgery, stabilisation, and hypoxia, ventilatory rate was 30–40 breaths/minute. Inspiratory time (0.4 second) and positive end expiratory pressure (4 cm H_2O) were kept constant throughout the experiment. Inspired fraction of O_2 and end tidal CO_2 were continuously monitored (Datex Normocap Oxy; Datex, Helsinki, Finland).

The right femoral artery was cannulated with polyethylene catheters (Portex PE-50, inner diameter 0.58 mm). Rectal temperature was maintained at 38–40°C with a heating blanket and a radiant heating lamp.

Experimental protocol
One hour of stabilisation was allowed after surgery. Hypoxaemia was achieved by ventilation with a gas mixture of 8% O_2 in N_2 (AGA, Oslo, Norway), until either mean arterial blood pressure reached 15 or base excess ≤ −20 mm Hg. CO_2 gas was added to produce a Pa_{CO_2} of 8.0–9.5 to imitate a situation of birth asphyxia. Before the start of resuscitation, the piglets were block randomised by the same person into six different groups by drawing lots. Resuscitation was performed with either ambient air (group A) or 100% O_2 (group B).

Each group was then further divided into three subgroups depending on ventilatory modus or resuscitation method. The piglets in group 1 (A1 n = 9/B1 n = 9) were hyperventilated, and had a low Pa_{CO_2} of 2.0–3.5 kPa. Group 2 (A2 n = 10/B2 n = 10) was resuscitated in a normal ventilatory modus (Pa_{CO_2} of 4.5–6.0 kPa). The animals in group 3 (A3 n = 10/B3 n = 10) had raised CO_2 level to reflect the situation of hypoventilation (Pa_{CO_2} 8.0–9.5 kPa). Group 3 was normoventilated but CO_2 gas was added. To hyperventilate the piglets (group 1), PIP and ventilatory rate were increased, and adjusted according to end tidal CO_2 and blood gases. The piglets were resuscitated for 30 minutes and then observed for another 150 minutes with normal CO_2. There were 18 piglets in group 1, and 20 piglets in each of groups 2 and 3 (n = 58).

Blood samples
Blood samples for analysis of blood gases were drawn from the femoral artery after surgery, at baseline (start of hypoxia), regularly throughout hypoxia, at the start of reoxygenation, at 10, 20, 30, and 120 minutes of reoxygenation, and at the end of the experiment. Temperature corrected acid/base status was measured with a Blood Gas Analyzer 860. Haemoglobin was measured at baseline (Co-oximeter 270). Blood samples for analysis of cTnI, creatine kinase-myocardial band (CK-MB), and myoglobin were collected in serum separator tubes at baseline and the end of the study, centrifuged, and kept at −70°C until analyses were performed (Access Immunoassay Systems; Beckmann Instruments, Nycopartner as, Norway).

At the end of the experiment, the piglets were given an overdose of 150 mg/kg pentobarbital intravenously. The heart was immediately removed; right and left ventricles were frozen in liquid nitrogen, and then kept at −70°C for further studies.

Statistical analysis
Continuous variables are given as mean (SEM). because of skewed distribution of cTnI, these variables were logarithmically transformed. To study the relation between cTnI, myoglobin, and CK-MB as dependent variables, and O_2 and CO_2 as independent variables, univariate analysis of variance was used. To accommodate multiple comparisons, the p values were adjusted according to Bonferroni. $p < 0.05$ was considered significant.

RESULTS
There were no significant differences between the groups with respect to number of animals, body weight, age, sex, rectal temperature, or time of hypoxaemia (table 1). Further, at the beginning of the experiment, there were no differences between blood pressure (mean (SEM) 74 (5) mm Hg, for each group; table 2), heart rate (mean 145 (4) beats/min), haemoglobin, serum glucose, or acid/base status between the groups. Blood pressure (fig 1), base excess, and heart rate were nearly normalised in all the groups at the end of the study (table 2).

Mean cTnI was increased more than 10-fold ($p < 0.001$) (table 3, figs 2 and 3). The release of cTnI in the hypoventilated group (high CO_2, group 3) was increased more than in the normoventilated group (normal CO_2, group 2) (NS). A similar pattern was seen in the hyperventilated group (low CO_2, group1). Group A (resuscitated with ambient air) showed a trend towards lower enzyme release than group B (resuscitated with 100% O_2) (NS) (fig 3).

CK-MB concentration was also increased (baseline 3.1 (0.6) µg/l; end point 5.2 (0.9) µg/l) towards the end of the experiment. A similar pattern was observed for myoglobin (baseline 6.7 (0.7) µg/l; end point 14.0 (1.7) µg/l). However, the concentrations of CK-MB and myoglobin released were very varied.

DISCUSSION
The release of cTnI confirms severe myocardial damage in this porcine asphyxia model. All the piglets, independent of ventilatory modus or treatment with oxygen or ambient air, showed a significant time dependent change in the concentration of cTnI. Whether resuscitation was with ambient air or 100% O_2 made no difference to the outcome. This is despite the fact that, in neonates and newborn animals, pulmonary vasodilation follows hyperoxaemia or oxygen treatment.[10] [19] [20]

Table 1 Baseline values

Group	CO₂ level	No of piglets	Weight (kg)	Hb (g/l)	Hypoxaemia duration (min)	BE (mmol/l)	pH
A 21% O₂	Low	9	1.9 (0.1)	7.6 (1.1)	63 (6)	1.71	7.45
	Normal	10	1.6 (0.1)	7.8 (2.2)	75 (10)	0.52	7.45
	High	10	1.6 (0.1)	8.4 (1.3)	56 (8)	1.68	7.42
B 100% O₂	Low	9	1.8 (0.1)	7.0 (1.3)	61 (7)	0.68	7.45
	Normal	10	1.7 (0.1)	8.0 (1.3)	65 (5)	0.10	7.43
	High	10	1.7 (0.1)	7.6 (1.6)	67 (11)	0.28	7.43

Values are mean (SEM).
BE, Base excess.

Table 2 Mean arterial pressure (MAP) and heart rate

Group	CO₂ level	MAP (mm Hg)			Heart rate (beats/min)		
		Baseline	Start resusc	End point	Baseline	Start resusc	End point
A 21% O₂	Low	72 (4)	23 (3)	50 (4)	140 (9)	185 (8)	183 (14)
	Normal	71 (6)	21 (3)	46 (5)	155 (6)	184 (17)	167 (10)
	High	74 (6)	23 (3)	47 (4)	137 (10)	170 (8)	144 (5)
B 100% O₂	Low	78 (3)	20 (2)	51 (4)	157 (5)	197 (11)	180 (9)
	Normal	74 (4)	26 (5)	52 (5)	141 (10)	194 (10)	142 (6)
	High	71 (4)	20 (2)	53 (3)	145 (12)	209 (21)	162 (7)
All groups		74 (5)	22 (2)	50 (3)	145 (4)	184 (6)	162 (4)

Values are mean (SEM).

From this, one could expect that reduced pulmonary artery pressure (PAP) would follow a decrease in pulmonary vascular resistance (PVR), with a secondary decrease in right ventricular workload.[21] We did not, however, find any evidence of reduced myocardial enzyme release in the piglets treated with 100% oxygen (fig 3). Our results showed a trend in the opposite direction. In all the piglets resuscitated with 100% O_2 (group B1, B3), except the normoventilated ones, the increase in cTnI release was greater than in the piglets resuscitated with ambient air (NS) (figs 2 and 3). Other mechanisms are thought to influence the outcome. Further investigations are necessary to resolve this.

In newborn piglets, as in neonates, asphyxia is one of the mechanisms that may induce pulmonary vasoconstriction and increased PVR. PVR is also augmented by acidosis and

hypercapnia.[20][22] Medbo et al[23] studied early changes in pulmonary haemodynamics during hypoxia and reoxygenation in normoventilated newborn piglets. They found that PAP increased significantly in the first few minutes, and then decreased at the end of the hypoxaemic period. During early reoxygenation, PAP rapidly increased significantly above baseline, before reaching values comparable to baseline. Fugelseth et al[24] examined pulmonary haemodynamic changes in unsedated piglets in a long term follow up study. They confirmed the early transient increase in PAP, assessed by echocardiography three hours after global hypoxic-ischaemic brain injury. The use of different models—that is, anaesthetised versus unsedated piglets—is probably one reason for the different timing of PAP normalisation. The different ages may also be important: the piglets in the experiment of Fugelseth et al were aged 12–36 hours, whereas those in the other group were 3–5 days old. Susceptibility to alterations in PVR may be greater closer to birth.

This model has been used previously to study cellular effects on other organs during hypoxaemia and reoxygenation.[23][25][26] With respect to the myocardium, a haemodynamic approach is necessary as well as cellular mechanisms. With regard to blood pressure (fig 1, table 2), all the piglets followed the same pattern. The large decrease suggested cardiac involvement and ventricular dysfunction. Blood pressure, however, has a multifactorial regulation. The piglets
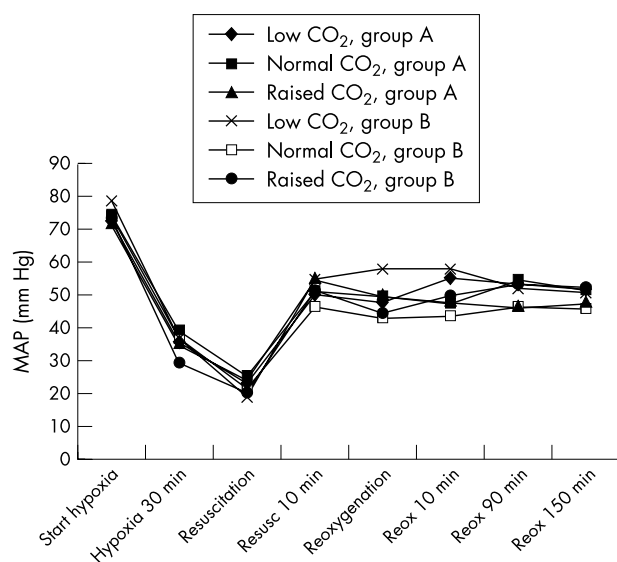

Figure 1 Mean arterial pressure (MAP). During hypoxaemia blood pressure decreased significantly, and during resuscitation it rose ending in a plateau about 20 mm Hg lower than at the start of the experiment.

Table 3 Cardiac troponin I (cTnI) concentration at baseline and end point

Group	CO₂	cTnI at baseline (µg/l)	cTnI at end point (µg/l)	Ratio
A 21% O₂	Low	0.115 (0.02)	2.340 (0.70)	20
	Normal	0.077 (0.02)	1.074 (0.24)	14
	High	0.087 (0.03)	1.771 (0.61)	20
B 100% O₂	Low	0.092 (0.01)	3.109 (1.32)	34
	Normal	0.114 (0.03)	1.506 (0.40)	13
	High	0.091 (0.01)	3.416 (1.49)	38

Values are mean (SEM). Ratio between cTnI levels describes the difference between the groups (NS).
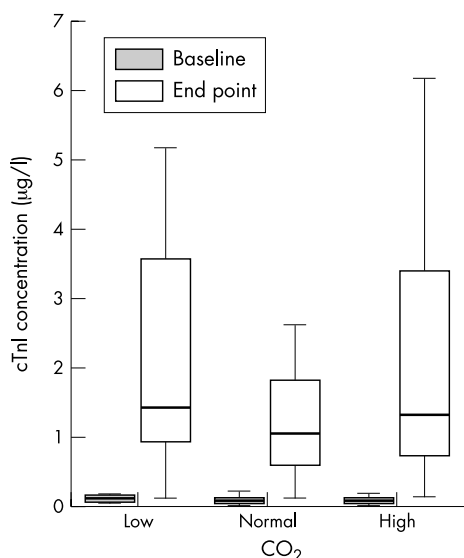
Figure 2 Cardiac troponin I (cTnI) related to CO_2. cTnI release was significantly ($p < 0.001$) increased in all three groups resuscitated by the different ventilatory modes. cTnI release was lower in the normoventilated piglets than the piglets with high or low CO_2 (NS).

in each group were given equivalent fluid therapy, 20 ml/kg throughout the experiment. None of the piglets were supported with inotropic drugs. Glucose concentration was kept within the selected range.

With respect to base excess, the piglets showed similar patterns, with a large decrease during hypoxia, a slower increase during resuscitation, and near normalisation at the end of the experiment. None of the animals were acid/base corrected.

In this study, the piglets with low or high CO_2 showed a greater release of cTnI than the piglets in the normoventilated group (group 2, fig 2), suggesting more serious cardiac deterioration. The piglets in group 3, with high CO_2, were normoventilated and supplied with CO_2 gas. Raised CO_2 concentration leads to vasoconstriction in the lungs and increased PVR and PAP. To achieve the low Pa_{CO_2} in the

hyperventilated groups (group 1), PIP and ventilatory rate were both altered during the 30 minutes of resuscitation. The considerable increase in PIP leads to increased intrathoracic pressure, which again may cause increased pressure in the pulmonary artery and increase the right ventricle workload.[21] Several studies have shown the effect of increased intrathoracic pressure on preload. Reduced venous return to the right ventricle is one of the mechanisms.[21] Low inspiratory CO_2 concentration leads to pulmonary vasodilatation. The decrease in Pa_{CO_2}, however, seems to play a minor role related to the large increase in intrathoracic pressure. The decrease in CO_2 is not capable of compensating for the effects of the PIP induced increase in intrathoracic pressure. The piglets going through a period of hyperventilation also had a higher heart rate than the other piglets at the end of the experiment ($p < 0.01$). In this group, heart rate also normalised, but at a lower rate. Increased heart rate reduces the time for coronary perfusion, which affects the myocardium, and may further increase myocardial susceptibility to impairment.

Serum CK-MB and myoglobin concentrations were variable. These proteins are present in striated muscle as well as the myocardium.[27] Blood samples were taken at baseline, about one hour after surgery, and at the end of the experiment. We suggest that, in addition to myocardial injury, the increase in the release of these proteins is the result of damage to the striated muscle caused by surgery. Previous reports have concluded that serum CK-MB is not a cardiospecific marker,[28–31] and neither is myoglobin.[27] Our study confirms that neither CK-MB nor myoglobin is a reliable marker of pure myocardial damage in neonates.

CONCLUSION
All groups in this hypoxia/reoxygenation model showed a significant release of cTnI, confirming severe cardiac dysfunction. Both haemodynamic and cellular mechanisms play a major part in cardiovascular regulation and influence the myocardial dysfunction caused by ischaemia-reoxygenation injury. Reoxygenation with 100% oxygen offered no biochemical protection against troponin release compared with ambient air. Normoventilation tended to produce a better myocardial outcome than hyperventilation or hypoventilation, as assessed by enzyme release.

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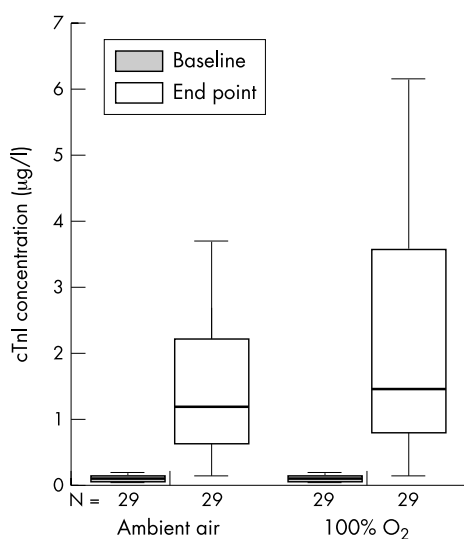

Figure 3 Cardiac troponin I (cTnI) related to O_2. There was an increase in cTnI from baseline to end point ($p < 0.001$) confirming serious cardiac involvement. There were no significant differences between the groups resuscitated with ambient air and 100% O_2.

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