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Consequences of Neonatal Resuscitation with Supplemental Oxygen

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The transition from fetal to neonatal life is frequently accompanied by a need for resuscitation with assisted ventilation. Guidelines issued by the American Academy of Pediatrics for neonatal resuscitation have traditionally recommended the use of 100% oxygen [O₂]¹. Over the past two decades the presumed benefits of pure O₂ under these conditions have begun to be challenged^{2,3}. Recent recommendations are proposing a role for resuscitation with less than 100% O₂, and intermediate values have been investigated⁴, although the practical aspects and functional consequences remain to be determined⁵.

To date studies addressing the effect of assisted ventilation with supplemental O₂ have been predominantly performed in human neonates. Piglets have been employed to evaluate the effects of hyperoxic exposure on molecular and biochemical markers of lung and brain injury^{6,7}. However, the piglet model presents limitations, in particular its relative maturity at birth and difficulty evaluating longer-term outcome measures. Therefore, we have developed a rat pup model to characterize physiologic and biochemical aspects of neonatal resuscitation with various concentrations of inspired O₂ as an adjunct to human studies.

Part I: Physiologic and Biochemical Consequences of Hyperoxic Resuscitation in Rat Pups

Onset of spontaneous respiratory efforts

Studies in human infants have shown a delayed onset of both first cry and sustained respiratory effort when resuscitation is performed with 100% vs 21% O₂^{3,5}. To our knowledge, no animal studies have been performed to investigate time to onset of spontaneous respiratory activity following central apnea induced by hypoxic depression of

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breathing. This would provide quantifiable data not readily available from human clinical trials. We hypothesized that both 21% and 40% O₂ would shorten the term to onset of spontaneous respiratory activity when compared to 100% O₂.

In anesthetized 8–10 day old rat pups [22 g], stainless steel EMG electrodes were inserted through the abdomen to record diaphragmatic activity. Following endotracheal intubation, the study protocol illustrated in Figure 1 was employed⁸. Time to onset of diaphragmatic activity was measured from the beginning of the resuscitation period with either 100% or 21% O₂ study gas. The same protocol was employed to compare 100% and 40% study gas for resuscitation.

Summary data for mean time to onset of spontaneous diaphragm activity are presented in Figure 2. A significant difference was found in the time to onset of spontaneous respiration between the room air [36±21 seconds] and 100% O₂ [72±22 seconds] groups. This difference was independent of the order of gas exposure. In contrast, there was no difference found between the use of 40% O₂ [84±27 seconds] and 100% O₂ [76±23 seconds].

Additional rat pups were studied using the same experimental protocol as for the physiologic study to obtain blood gas data via carotid artery sampling. Comparison of blood gas status after 30 s exposure to each study gas is shown in Table 1. As expected, there was an overall increase in PaO₂ [p<0.0001, ANOVA] and SaO₂ [p<0.0001, ANOVA] corresponding to the increase in O₂ concentration of the study gas [Table 1]. There was no effect of supplemental O₂ on pH, however, there was a decrease in PaCO₂ [p<0.05, ANOVA] and bicarbonate [p<0.05, ANOVA] in pups resuscitated with supplemental O₂.

Our findings confirm those of available clinical studies comparing 100% O₂ versus room air resuscitation and demonstrate a delayed onset of spontaneous breathing in the hyperoxic environment. In addition, we have examined an intermediate level of supplemental O₂, 40%, compared its efficacy to that of the current standard of 100% O₂, and found no difference between these two gases with regards to onset of respiratory activity.

It is well known that neonatal exposure to hypoxia results in a biphasic respiratory response⁹. This comprises an initial excitation of respiration secondary to excitation of peripheral chemo receptors, followed by a central depression of respiration. We used exposure to 5% O₂ to elicit apnea in our model as low O₂ tension probably contributes to inhibition of fetal respiratory activity and the respiratory depression during perinatal asphyxia. While hypoxic exposure always elicited apnea, there was variability in the time from onset of hypoxic exposure to onset of apnea. Therefore, we continued exposure with 5% O₂ for an additional 90 s to standardize the protocol between rat pups, as illustrated in Figure 1.

During hypoxia, sustained increase in peripheral chemoreceptor drive will oppose central depression of respiration. Hence, hyperoxia during resuscitation should suppress peripheral chemoreceptor drive and delay re-initiation of breathing. This was first proposed by Dejours and, more recently, adapted for the infant in studies by Bouferrache et al^{10,11}. We hypothesized this is to be a likely mechanism for the delayed onset of respiration when resuscitation with 100% O₂ is compared with the use of 21% O₂. Our blood gas results indicate that, as with 100% O₂, 40% O₂ also creates a hyperoxemic environment, explaining the similar time to onset of respiratory activity when compared with 100% O₂. It is interesting to note quantitative similarities regarding onset of respiratory activity between the current data and human infant data from Saugstad and the RESAIR 2 Study². Saugstad observed that the first breath occurred one minute after beginning resuscitation with room air and after 1.5 minutes when 100% was used. We also found a difference of approximately 30 s between the onset of respiratory efforts when 21% and 100% O₂ were compared.

Factors other than blood gas status [e.g., sensory stimuli or chemical mediators] have been proposed to contribute to onset of continuous breathing after birth. Hertzberg et al showed that arterial chemo receptors are active and responsive in the fetal lamb, but that their sensitivity is adjusted to the low fetal arterial O₂ tension and that after birth the chemo receptors become silent and gradually reset their sensitivity¹². While our blood gas data revealed an anticipated incremental increase in PaO₂ with increasing supplemental O₂ resuscitation, we did not predict that supplemental O₂ during resuscitation would result in the observed decrease in PaCO₂. This may occur via more rapid relief of pulmonary vasoconstriction in a hyperoxic environment, and enhanced CO₂ elimination. The possibility that hypocapnia, during resuscitation with supplemental O₂, contributed to the delayed onset of breathing cannot be excluded as hyperoxemia and hypocapnia occurred in both groups resuscitated with supplemental O₂. Further study is clearly appropriate to document whether hypocapnia is consistently associated with hyperoxic resuscitation. Such hypocapnia, if severe, could have potential detrimental effects on cerebral blood flow.

We recognize several reservations regarding our experimental model. Blood gases collected at the end of the apneic period before resuscitation were consistent with hypoxic respiratory depression but showed no evidence of metabolic or respiratory acidosis as one would expect in asphyxiated newborns. We did attempt to replicate CO₂ accumulation by adding CO₂ concentrations as low as 1% to the hypoxic gas that was used to induce apnea, however, use of a hypercapnic, hypoxic gas failed to induce apnea and, thus, we were unable to conduct our experiments. Another limitation of our model was that the experiments were not performed during the actual transition from fetal to neonatal life. While we chose a rat pup model due to the animal's relative immaturity at birth, our experiments took place eight to 10 days after birth at a time when the animal's maturation approaches term gestation in the human. This postnatal age was chosen because of our ability to readily intubate and ventilate the rat pups at and beyond eight days of age. Future studies might focus on comparable resuscitation protocols in rat pups closer to the transition from fetal to neonatal life.

We conclude that our novel rat pup model allows an accurate assessment of the relative efficacies of 100% O₂, 40% O₂ and room air in reinitiating respiratory efforts following apnea. Our studies show that under conditions of mild hypocarbia even modest levels of supplemental O₂, such as 40% O₂, cause hyperoxia and delay the onset of spontaneous respiratory efforts. The latter may be clinically relevant if it results in a prolonged duration of ventilatory support, so potentially predisposing to morbidity associated with neonatal lung injury.

Markers of oxidant stress

While onset of spontaneous breathing is clearly influenced by resuscitative gas, short and longer term biochemical measures of oxidant injury in various organ systems are of potentially far greater significance. While human trials are key, animal models, again, may provide the ability to assess biochemical outcomes not readily attainable in human infants. While commonly used rat or murine models of asphyxia have proven useful^{13,14}, they employed carotid artery ligation and thus differ from clinical asphyxia. We hypothesized that rat pups exposed to hypoxia and resuscitated with 100% O₂ would differ in biochemical markers of oxidant injury when compared to normoxia-resuscitated pups.

In this protocol we studied 12-day old rat pups that were anesthetized, intubated, and ventilated with a tidal volume of 0.01 cc/gm body weight at a rate of 60 breaths per minute to maintain normocapnia¹⁵.

To determine the degree and duration of hypoxia necessary to demonstrate production of cortical lactate, rat pups were randomized to be ventilated with 21% or 5% O₂ for up to 60

minutes. At each time point, beginning after five minutes exposure to 21% or 5% O₂, a whole-brain freezing method of fixation¹⁶ with liquid nitrogen was performed and the frontal cortices of the pups were dissected and kept at -80°C. Lactate concentration in tissue, as a measure of cellular energy metabolism, was determined by standard luciferin-luciferase bioluminescence and biochemical assays, respectively, as previously reported¹⁶. As seen in Figure 3, in which each data point represents one sacrificed pup, 5% O₂ exposure was accompanied by lactate accumulation in brain tissue. We, therefore, chose a 15-minute hypoxic exposure for our subsequent protocol as death from 5% O₂ inhalation occurred at approximately 17 minutes in 50% of the rat pups [Figure 3].

We then sought to determine the effect of resuscitative gas on disruption of oxidative metabolism. A non-intubated, non-ventilated group [n=6] of rat pups served as controls. The other three groups were sedated, intubated, and stabilized. Normoxia ventilated pups [n=18] were neither asphyxiated nor resuscitated and remained in 21% O₂. Based on the results from the previous experiment, and as described in Figure 4, experimental animals were asphyxiated with 5% O₂ for 15 minutes and further randomized to resuscitation with 21% O₂ [RAR] [n=18] or 100% O₂ [OxR] [n=18] for 30 minutes.

Blood was then collected by cardiac puncture for sampling at one of two randomized time points. The first group of pups was sampled immediately following the 45 minutes of experimental or control conditions [T₀]. A second group of pups was weaned from the ventilator, returned to their dams, and re-sedated and sampled 24 hours later [T₂₄].

Total glutathione was determined in whole blood by high performance liquid chromatography [HPLC] of the dinitrophenol derivative following the method described by Fariss and Reed¹⁷. Oxidized glutathione [GSSG] was determined by HPLC using N-ethylmaleimide as a thiol chelant agent to avoid glutathione oxidation during sample processing, as described previously by our group¹⁸. Reduced glutathione [GSH] levels were calculated as follows: GSH - total glutathione - 2xGSSG.

GSSG levels increased immediately after hypoxia and resuscitation in the room air-resuscitated group compared to controls, as well as in the oxygen-resuscitated group compared to controls. No significant differences were found between room air-resuscitated and oxygen-resuscitated groups. Blood GSSG levels returned to control values 24 hours after resuscitation with 21% or 100% O₂.

Immediately following hypoxia and resuscitation with 21% or 100% O₂, there were no differences in GSH levels among the room air-resuscitated group, the oxygen-resuscitated group, or non-asphyxiated controls. Twenty-four hours later, however, GSH levels were significantly decreased after resuscitation with pure oxygen compared with non-asphyxiated control rats. GSH levels were significantly decreased in the oxygen-resuscitated group compared with the room air resuscitated group. There remained no difference between the room air-resuscitated group and controls [Figure 5].

Employing our rat pup model, we have now demonstrated that ventilation with 5% O₂ for 15 minutes caused intense tissue hypoxia, as reflected in elevated lactate levels (Fig. 6). After re-oxygenation, elevated levels of GSSG were found regardless of whether the pups were resuscitated with O₂ or room air, and this immediate response of GSSG is consistent with data from human infants³. In contrast to human infant data, GSSG levels returned to basal levels 24 hours after resuscitation with 100% or 21% O₂. This rapid recovery requires further investigation and may have explanations beyond the high metabolic rate noted in rodents. However, blood GSH levels were significantly lower 24 hours after resuscitation with 100% O₂ compared with 21% O₂. GSH is the major thiolic antioxidant in cells and, hence, its depletion would reflect exhaustion of the antioxidant defense upon the use of pure

O₂^{19,20}. An increase in hyperoxia-induced oxygen free radicals would lead to consumption of this critical antioxidant agent.

There are several limitations to our model that need to be acknowledged, including the absence of hypercarbia and the age of the animals as noted earlier. However, it should be noted that 12-day-old, Sprague-Dawley rat pups have many neuron-anatomical and neurodevelopmental similarities with the term neonate²¹. Of note is the ability to asphyxiate and resuscitate rat pups and return them to their mother for rearing, which allows evaluation of both the short and long-term effects of hyperoxia on the developing animal. This may very well contribute to our understanding the immediate effects of hyperoxia, as well as its long-term consequences on the resuscitated newborn infant.

Part II: Implications for Hyperoxic Resuscitation in Human Infants

Background

Fetal life elapses in a low oxygen environment with a mean intrauterine arterial oxygen saturation [SpO₂] under physiologic conditions of 40–45%. In the first minutes of life an abrupt rise of SpO₂ to 80–90%^{22, 23, 24} will cause a physiologic pro-oxidant situation which facilitates activation of specific metabolic pathways²⁵. However, under pathological conditions, such as birth asphyxia, a series of pathophysiologic events will lead to a severe oxidative stress which may cause tissue damage^{26,27}. Thus, repeated episodes of hypoxia cause purine derivatives such as adenosine or hypoxanthine to accumulate and promote specific changes that predispose cells to enhanced damage upon re-oxygenation²⁸. Activation of oxidases and iNOS, and up-regulation of HIF 1 α , as well as down-regulation of antioxidant enzymes, such as superoxide dismutases, catalases, and glutathione peroxidases, will together generate a burst of reactive oxygen [ROS] and nitrogen species [RNS] upon re-oxygenation^{28,29,30} (Figure 7). Accumulation of free radicals within the mitochondria will trigger the c-aspartate proteases (caspases) pathway leading to apoptosis thus amplifying initial damage caused by necrosis³¹.

In the human neonate prospective studies performed over the last 15 years comparing the use of room air versus pure oxygen resuscitation have confirmed these experimental findings, and triggered a debate as to how oxygen should be managed during resuscitation of asphyxiated newborn infants³². In addition, very recent studies have also approached the feasibility of using low oxygen concentrations in extremely low birth weight infants [ELBW]^{33,34}. This second part will deal with the metabolic and clinical consequences of use of 100% or 21% oxygen in the resuscitation of asphyxiated neonates, and underscore recent results regarding resuscitation of ELBWs with low or high oxygen concentrations.

Use of room air versus 100% oxygen in the term and near term infant

a) Clinical recovery—Several prospective clinical trials have shown that asphyxiated newborn infants resuscitated with room air exhibited higher one-minute Apgar score than newborn infants resuscitated with 100% oxygen. Moreover, although no significant difference was found in five-minute Apgar scores between groups, babies in the 100% oxygen group had consistently lower scores than those in the room air group. In addition, in these studies room-air resuscitated infants consistently initiated a spontaneous pattern of respiration and exhaled first cry earlier than babies in the pure oxygen group^{2,3,35,36,37}. However, other studies failed to show significant differences for both Apgar score or for time needed for the onset of the first cry or spontaneous respiration between both groups^{38,39} (Figure 8). Finally, no differences in heart rate were found between ambient air or 100% oxygen resuscitated groups in every performed study.

Serial arterial blood gases performed during resuscitation in babies ventilated with higher oxygen concentrations showed $\text{PaO}_2 > 100$ torr at the end of resuscitation, and also when clinical recovery was achieved, while babies ventilated with room air always had a PaO_2 in the range of 80–90 torr³⁶ (Figure 9). As shown in our experimental rat model described earlier, administration of high oxygen during resuscitation is associated with delayed diaphragm activation as compared to the use of lower oxygen, and especially room air. As a consequence the use of pure oxygen can cause a prolongation of resuscitation of up to several minutes, and implies the administration of an additional 350 mL of pure oxygen to babies in the 100% oxygen group³⁶. No differences between babies resuscitated with room air or pure oxygen regarding pCO_2 or base excess have been found, although our rat pup data suggest hypocapnia may result during hyperoxic resuscitation. Peripheral chemo receptors are sensitive to arterial blood oxygen content⁴⁰, therefore, hyperoxemic infants resuscitated with 100% oxygen could exhibit blunted chemoreceptor triggering responses and delayed onset of respiratory drive. Interestingly enough, Saugstad et al., did not find differences in arterial oxygen saturation as measured by pulse oximetry [SpO_2] in the first minutes of life in asphyxiated babies resuscitated with different gas mixtures⁴¹. This can be partially explained by the shape of the hemoglobin saturation curve. Thus, in the steep part of the curve small changes in PaO_2 cause great changes in SpO_2 , while in the higher saturation ranges the curve shows a horizontal shape, therefore similar SpO_2 may correspond to significantly different PaO_2 values.

b) Mortality—In recent years three systematic reviews and a meta-analysis comparing resuscitation with ambient air and 100% oxygen have been published^{32,42,43}. The most consistent item addressed has been neonatal mortality [deaths before 7 days after birth], although other important outcomes, such as long-term neurodevelopment, secondary signs consistent with hypoxic ischemic encephalopathy, delay in the onset of regular respiration or achievement of heart rate > 100 bpm, and Apgar scores, were also analyzed. Davis and co-workers⁴² studied five trials using the methods and software of the Cochrane Collaboration. Altogether 1302 babies pertaining to three unmasked and two masked studies fulfilled the inclusion criteria. Although no trial individually showed a difference in mortality, the pooled analysis showed a significant benefit for infants resuscitated with room air [relative risk 0.71 (95% CI 0.54 to 0.94), risk difference -0.05 (-0.08 to -0.01)]. The effect of ambient air versus 100% oxygen upon long-term development could not be determined reliably because of methodological limitations of the studies. The authors concluded that, for term and near term newborn infants, air should be used initially with oxygen as back up if initial resuscitation failed⁴².

Saugstad and co-workers⁴³ systematically reviewed the literature that included searching Medline/PubMed/EMBASE and the Cochrane Review Library databases for randomized or pseudo-randomized, blinded or not, studies of depressed newborn infants resuscitated with 21% or 100% oxygen. Although this meta-analysis comprised the same five trials as the one by Davis et al, it included a greater number of infants because the authors had direct access to the trial databases. Thus, 1737 depressed newborn infants were included, 881 resuscitated with room air, and 856 with 100% oxygen. Neonatal mortality was 8.0 vs 13.0 in the 21% and 100% oxygen groups, respectively, OR 0.57, 95% CI 0.42–0.78. Moreover, in babies having a low Apgar score at one minute [< 4] no differences were found between the groups. In addition, Apgar scores at five minutes and heart rate at 90 seconds were higher, and time to first breath was significantly earlier in the ambient air group as compared to the pure oxygen group⁴². The authors concluded that neonatal mortality was significantly reduced when depressed newborn infants are resuscitated with 21% oxygen instead of pure oxygen, and recovery was faster in the ambient air group.

The most recent meta-analysis has been performed by Rabi and co-workers³². A systematic review of the literature was undertaken in August 2005 that included seven studies with a total of 2011 infants [2 additional studies were incorporated in the meta-analysis]. Compared to the 100% oxygen resuscitation group, neonates in the room air group had lower mortality both in the first week of life [OR 0.70, 95% CI 0.50 to 0.98] and at one month and beyond [OR 0.63, 95% CI 0.42 to 0.94] (Table 2). The incidence of severe hypoxic ischemic encephalopathy [Grade II & III] according to Sarnat & Sarnat classification⁴⁴ was similar between the groups. Rabi and co-workers concluded that room air resuscitation is superior to 100% oxygen as the initial choice for resuscitating clinically depressed newborn infants as it may result in a lower mortality rate³².

Although not conclusive, these studies provide evidence that use of high oxygen concentrations at birth for term or near term babies significantly increase mortality and morbidity. Therefore, high oxygen concentrations should be used with extreme caution in the perinatal period⁴⁵. In this scenario, several national and local guidelines now recommend starting with 21% oxygen and adding supplementary oxygen if the infant is not responding adequately within 90 seconds^{46,47}.

Resuscitation of extremely low gestational age neonates with low or high oxygen

Extremely low gestational neonates [ELGAN] frequently need respiratory support to adequately adapt to the extra uterine world. Different approaches to optimize ventilatory support have been the subject of numerous publications in recent years^{48,49,50,51}. However, very little information is available regarding the ideal proportion of supplemental oxygen needed to optimize fetal to neonatal transition and avoid oxidative stress and tissue damage. Wang and co-workers³³ performed a prospective randomized clinical trial with premature infants of 23 to 32 weeks' gestation assigning one group to room air [RA] and the other to 100% oxygen [O₂ group]. The O₂ group was initially ventilated with 100% oxygen and weaned after five minutes if SpO₂ was >95%. The RA group was initially ventilated with 21% oxygen and progressively increased to 100% in 25% increments if SpO₂ was <70% at three minutes or <80% at five minutes of life. A total of 41 babies were evaluated: 21 in the RA group and 18 in the O₂ group. Every neonate in the RA group met the rescue criteria and needed oxygen increments at three minutes of life; six patients were directly switched to 100% oxygen and the rest were adjusted with incremental increases. SpO₂ was significantly lower in the RA group from two to 10 minutes [p<0.001]. Thus, at three minutes, babies in the RA group had SpO₂ of 55% as compared to 87% for babies in the O₂ group. Heart rates did not differ between groups in the first 10 minutes of life, and there were no differences in secondary outcomes [IVH, ROP, NEC or CLD]. Wang and coworkers concluded that room air should not be used as the initial resuscitating gas for premature infants of less than 32 weeks' gestation.

In another study Dawson et al⁵² have compared short-term outcomes of resuscitation of preterm infants with 100% oxygen versus room air. In July 2006 the policy of the Royal Women's Hospital [Victoria, Australia] was changed from using 100% oxygen to air in the delivery room. This is a cohort study comparing two groups of infants or less than 30 weeks' gestation born before and after this change in policy. Sixteen infants initially resuscitated with pure oxygen and 27 with room air were included. For the 100% oxygen group the SpO₂ rose to a median of 78% at two minutes and 90% by five minutes. By eight minutes 50% had a SpO₂ 95%. In the RA group the median SpO₂ was 25% at two minutes and 65% at five minutes. Supplementary oxygen was started for 23 infants in the RA group at a median of 5.2 minutes; the SpO₂ rose to a median of 85% by six minutes; 26 had a SpO₂ 95% at eight minutes. No differences regarding heart rate in the first 10 minutes of life were evidenced. Thus, the authors concluded that 85% of the babies initially resuscitated with air

received supplemental oxygen; however, when using pulse oximetry to monitor and titrating supplemental oxygen, SpO₂ rose to within the normal range by six minutes of life⁵².

The last published study is a prospective randomized clinical trial performed in two regional reference centers in Spain [Madrid & Valencia] aiming to reach a target saturation of 85% at 10–15 minutes of life in extremely premature infants resuscitated initially with low oxygen [30%] or high oxygen [90%]³⁴. Preductal SpO₂ was continuously registered and FiO₂ adjusted in 10% intervals according to heart rate [HR >100 bpm] and pulse oximetry [SpO₂ >85%]. Inspired oxygen in the low oxygen group was increased stepwise to 45%, and in the high oxygen group reduced to 45% to reach a stable SpO₂ of approximately 85% at 5–7 minutes in both groups (Figure 10). No differences in SpO₂ were found, regardless of the initial inspired oxygen employed after four minutes of cord clamping. No differences in mortality in the early neonatal period were detected. The authors concluded that it is safe to initiate ELGAN resuscitation with a low oxygen concentration [~30%] and, thereafter, adjust according to clinical response³⁴.

These three studies conclude that with the present ventilatory strategies in the delivery room, it is not routinely recommended to use room air to successfully resuscitate ELGAN. Better results have been obtained using initially slightly higher inspiratory fractions of oxygen (e.g., 30%) and adjusting them thereafter to optimize the fetal to neonatal transition and successfully switch to room air. Thus, optimizing the use of pulse oximetry oxygen load administered to ELBW infants may be significantly reduced and subsequently the negative consequences of hyperoxygenation.

Future studies evaluating optimal ventilatory strategies and inspired oxygen adjustment criteria will contribute to improved short and long term outcome of these very special groups of infants.

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Figure 1.

Experimental design. During the control period, the pups were intubated, but not connected to a ventilator. During mechanical ventilation, the pups were initially ventilated with 5% O₂ to induce apnea, as illustrated by the cessation of diaphragmatic activity. They continued to be ventilated with 5% O₂ for an additional 90 sec and then were resuscitated with the study gas for 30 sec. The ventilator was then disconnected and the return of diaphragm EMG quantified [8]

Figure 2.

Summary data for time to onset of spontaneous respiration between experimental groups comparing 100% O₂ vs 21% O₂ and 100% O₂ vs 40% O₂. There was a significant difference [$p=0.002$] in comparing the 100% O group against 21% O₂, $n=10$. There was no difference between 100% O₂ and 40% O₂, $n=11$ [8].

Lactate Concentration in Asphyxiated Rat Pups

Figure 3. Cortical lactate accumulation after sedation and ventilation of rat pups with 21% or 5% oxygen for up to 60 minutes. Each data point represents one sacrificed animal at that time. Control pups are non-intubated and non-ventilated.

Protocol

Figure 4.

Experimental design. A non-intubated, non-ventilated group [n=6] served as controls. All the other groups were sedated, intubated, and stabilized for 5 minutes. Normoxia ventilated pups [n=18] were neither asphyxiated nor resuscitated and remained in 21% oxygen for 45 minutes. Experimental groups were ventilated for 15 minutes with 5% oxygen to induce hypoxemia and then randomized to reoxygenation with 100% [OxR] [n=18] or 21% [RAR] [n=18] for 30 minutes. Blood samples in all groups were then collected immediately [T₀] or 24 hours after recovery [T₂₄].

GSSG in Resuscitated Rat Pups

Figure 5.

Whole blood oxidized glutathione levels [GSSG] after 15 minutes of asphyxia of rat pups with 5% oxygen followed by 30 minutes resuscitation with 21% [RAR] or 100% oxygen [OxR]. GSSG levels increased significantly in both resuscitated groups compared to controls, with return to control after 24 hours.

GSH in Resuscitated Rat Pups

Figure 6.

Whole blood reduced glutathione [GSH] after 15 minutes of asphyxia of rat pups with 5% oxygen followed by 30 minutes resuscitation with 21% [RAR] or 100% oxygen [OxR].

GSH levels did not initially differ between resuscitated and control groups, however, at 24 hours GSH was significantly decreased in the 100% oxygen resuscitated group.

Figure 7.
Patho-physiology of asphyxia –reoxygenation [Ref #27]

Figure 8. Time needed for the onset of a regular pattern of respiration (mean $\pm$ SD)

Figure 9.
Partial pressure of Oxygen in asphyxiated babies resuscitated with room air or 100% oxygen in the first 15 minutes of life.

Partial pressure of oxygen in asphyxiated newly born infants resuscitated with room air (RAR) or 100% oxygen (OxR)

Figure 10.

Oxygen inspiratory fraction (FiO₂) needed to achieve a target saturation (SpO₂) of 85% in the first 10 minutes of life in extremely low gestational age neonates initially ventilated with low (30%) or high (90%) oxygen concentration.

TABLE 1

Blood Gas Status Following 30 Seconds Resuscitation with Study Gas

	21% O₂ (n=6)	40% O₂ (n=6)	100% O₂ (n=6)
pH	7.37 ± [1.15×10 ⁻⁸]	7.45 ± [9.26×10 ⁻⁹]	7.37 ± [5.12×10 ⁻⁹]
PaCO₂ (mm Hg)	30 ± 9.1	19 ± 6.5 [†]	21 ± 3.5 [†]
PaO₂ (mm Hg)	82 ± 17	169 ± 23 [*]	233 ± 8 [*]
SaO₂ (%)	95 ± 2.7	99 ± 0.5 [*]	100 ± 0.4 [*]
HCO₃ (mmol/L)	17 ± 4 [§]	14 ± 4 [§]	11 ± 3 [§]

[†]p<0.05 versus 21%.^{*}p<0.01 versus 21%.[§]p<0.05 between groups

Table 2

Outcome of death at one week (early neonatal death). Odds ratio < 1 favors room air (Rabi Y et al Resuscitation 2007 [Ref # 31])

Study	Odds ratio (95% CI)	% weight
Ramji S et al (Pediatr Res 1993)	0.73 (0.15 – 3.49)	5.9
Saugstad OD et al (Pediatrics 1998)	0.69 (0.44 – 1.06)	78.7
Vento M et al (Biol Neonate 2001)	0.11 (0.01 – 0.90)	12.4
Vento M et al (Pediatrics 2001)	1.10 (0.06 – 18.77)	1.4
Vento M et al (J Pediatr 2003)	0.99 (0.06 – 16.07)	1.6
Overall	0.63 (0.42 – 0.94)	