

University of Groningen

Intestinal oxygenation, growth, and development of preterm infants

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DOI:
[10.33612/diss.199935955](https://doi.org/10.33612/diss.199935955)

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Document Version
Publisher's PDF, also known as Version of record

Publication date:
2022

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):
Dotinga, B. (2022). *Intestinal oxygenation, growth, and development of preterm infants*. [Thesis fully internal (DIV), University of Groningen]. University of Groningen. <https://doi.org/10.33612/diss.199935955>

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Chapter 3

Splanchnic oxygen saturation during reoxygenation with 21% or 100% O₂ in newborn piglets

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Pediatr Res. 2021. Online ahead of print

Abstract

Background: Increasing evidence recognizes the harm of excess oxygen to lungs, eyes, and brain of preterm infants, but not yet to the intestine. We assessed changes in splanchnic oxygenation during reoxygenation with 21% compared to 100% O₂ in a newborn piglet model of perinatal asphyxia.

Methods: We randomized 25 piglets to control or intervention. Intervention groups underwent global hypoxia until acidosis and hypotension occurred. Piglets were reoxygenated for 30 minutes with 21% or 100% O₂ and observed for 9 hours. We continuously measured regional splanchnic oxygen saturation (r_sSO₂) using near-infrared spectroscopy (NIRS). We calculated mean r_sSO₂ and r_sCoVar (as SD/mean). We measured PaO₂ and SaO₂, sampled from the right carotid artery.

Results: Reoxygenation after global hypoxia restored r_sSO₂. Reoxygenation with 100% O₂ increased r_sSO₂ to values significantly higher than baseline. In intervention groups, r_sCoVar decreased during observation compared to baseline. We found a correlation between r_sSO₂ and PaO₂ ($r=0.420$, $P<0.001$) and between r_sSO₂ and SaO₂ ($r=0.648$, $P<0.001$) in pooled data from the entire experiment.

Conclusion: Reoxygenation after global hypoxia improves splanchnic oxygenation, but is associated with reduced variability of r_sSO₂. Reoxygenation with 100% O₂ exposes the intestine to hyperoxia. Splanchnic NIRS is able to detect intestinal hypoxia and hyperoxia.

Introduction

In preterm infants, the mechanisms that regulate intestinal oxygenation may be immature, leading to a higher susceptibility to changes in oxygen supply and oxygen demand compared to term infants.¹ Therefore, preterm infants may frequently be exposed to intestinal hypoxia following changes from baseline conditions, such as enteral feeding, hypoxic hypoxemia, anemic hypoxemia, hypotension, and hypothermia.^{1,2} Intestinal hypoxia may compromise intestinal function and contribute to the development and progression of gastrointestinal diseases, including necrotizing enterocolitis (NEC).³ At the same time, increasing evidence recognizes the harmful effects of excess oxygen, that could trigger inflammation and oxidative stress.^{4,5} Therefore, the best resuscitation strategy for preterm infants in the delivery room remains unknown, although it seems a majority of very preterm infants require some amount of oxygen supplementation in the first 5 minutes to reach oxygen saturation targets.⁶ Nevertheless, preterm infants may be especially susceptible to oxidative stress due to immature antioxidant defenses.^{5,6} While oxygen toxicity has been documented for the lungs, eyes, and brain of preterm infants, the effects of hyperoxia on the intestine remain largely unknown.⁵

Monitoring of regional splanchnic oxygen saturation ($r_s\text{SO}_2$) using near-infrared spectroscopy (NIRS) has gained interest in the past decade and may be used to detect intestinal exposure to hypoxia and hyperoxia.⁷⁻⁹ NIRS is a non-invasive method that uses near-infrared light to measure the ratio of oxygenated to total hemoglobin (Hb) for the combined arterial, capillary, and venous sources in the underlying tissue.⁷⁻⁹ The vascular distribution is generally accepted to be approximately 20% arterial, 5% capillary, and 75% venous.^{8,9} Splanchnic NIRS monitoring was validated by correlating $r_s\text{SO}_2$ with blood flow velocity in the superior mesenteric artery (SMA) and with mixed venous oxygen saturation (SvO_2), portal vein oxygen saturation (SpvO_2), and umbilical vein oxygen (SuvO_2) in both animal models and neonates.¹⁰⁻¹⁴ A recent study by Chen et al. demonstrated that splanchnic NIRS monitoring can accurately detect changes in splanchnic oxygenation in a piglet model of intestinal hypoxia produced by graded hypoxia or SMA ligation.¹⁴ The response of $r_s\text{SO}_2$ to different resuscitation strategies following global hypoxia has not previously been studied, but it has been documented that $r_s\text{SO}_2$ increases in response to red blood cell (RBC) transfusions.¹⁵⁻²¹ In addition, changes in variability of $r_s\text{SO}_2$ after RBC transfusion have been suggested to contribute to transfusion-associated NEC.^{16,20,22} Nevertheless, the mechanisms leading to intestinal injury following anemic hypoxemia and RBC transfusion may differ from those following hypoxic hypoxemia and reoxygenation.

The aims of this study were to assess the effects of global hypoxia and different resuscitation strategies on mean $r_s\text{SO}_2$ and variability of $r_s\text{SO}_2$ and to verify that splanchnic NIRS monitoring can accurately reflect changes in splanchnic oxygenation in a newborn piglet model of asphyxia.

Methods

Study design

This study was part of a larger experiment that included 42 Noroc (LyxLD) piglets aged 6-36 hours. We excluded piglets with Hb levels <5.0 g/dL and signs of sickness. All piglets underwent anesthesia, ventilation, and surgical preparation according to previously described protocols in studies using this newborn piglet model of inflicted asphyxia.^{23,24} After surgery, piglets were stabilized during 1 hour and randomized to a control group or an intervention group. Piglets in the intervention groups were subjected to global hypoxia, achieved by ventilation with 8% O_2 in N_2 . We aimed to keep PaCO_2 between 8.0-9.5 kPa by adding CO_2 to mimic perinatal asphyxia. Hypoxia was continued until severe metabolic acidosis (arterial base excess <-20 mmol/L) or severe hypotension (mean arterial blood pressure <20 mmHg) occurred. Piglets were then reoxygenated for 30 minutes according to randomization, i.e., 21% O_2 , 100% O_2 , or 100% O_2 for 3 minutes followed by 21% O_2 for 27 minutes. We will continue to refer to these intervention groups as “21% O_2 ”, “100% O_2 (30’)”, and “100% O_2 (3’)”, respectively. The latter group was added, as a limited period of reoxygenation with 100% O_2 may rapidly correct hypoxia, yet prevent prolonged exposure to hyperoxia. Piglets were observed for 9 hours after reoxygenation. We chose this duration for observation to partially include the secondary energy failure that starts approximately 6 hours after the initial incident.²⁵ During this period of observation, piglets were ventilated with 21% O_2 . At the conclusion of the experiment, piglets were euthanized using an overdose of pentobarbital. Apart from hypoxia and reoxygenation, piglets in the control group underwent the same experimental setup. An overview of the study protocol is presented in Figure 1. The experimental protocol has been approved by the Norwegian Council for Animal Research. Animals were handled in accordance with European Guidelines for Use of Experimental Animals, by researchers certified by the Federation of European Laboratory Animal Science Associations (FELASA).

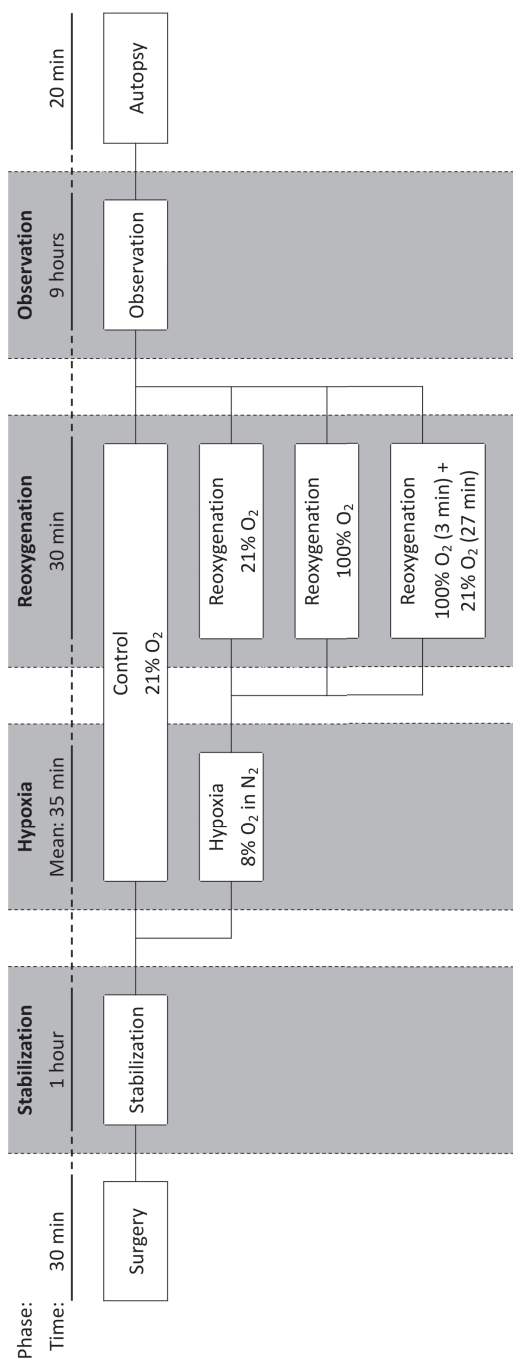


Figure 1. Study protocol.

Measurements

Throughout the study, we monitored heart rate, blood pressure, peripheral oxygen saturation (SpO_2), and rectal temperature. We performed regular arterial blood gas analyses for temperature-corrected arterial acid-base status, arterial oxygen content, lactate, glucose, and Hb, sampled from the right carotid artery. In addition, we continuously monitored $r_s\text{SO}_2$ with NIRS using an INVOS™ 5100C monitor (Medtronic, Dublin, Ireland) and a neonatal INVOS™ SomaSensor placed in the right lower quadrant of the abdomen, to target the terminal ileum and to avoid the bladder.¹⁴ We calculated mean $r_s\text{SO}_2$ and the coefficient of variation for $r_s\text{SO}_2$ ($r_s\text{CoVar}=\text{SD}/\text{mean}$), as a measure of variability of $r_s\text{SO}_2$. We used 5-minute periods to pool and average $r_s\text{CoVars}$.²⁶

Statistical analyses

For analyses, we divided the study in four phases, that are referred to as “stabilization”, “hypoxia”, “reoxygenation”, and “observation” (Figure 1). Given the long duration of observation, we analyzed this phase per hour. First, normality of the data was assessed using the Kolmogorov-Smirnov test. Next, we assessed whether mean $r_s\text{SO}_2$ changed during the study compared to stabilization within groups using a Friedman repeated-measures analysis of variance by ranks test, with post hoc comparison to stabilization. To gain more insight into exposure to prolonged hypoxia and exposure to hyperoxia after the initial incident, we analyzed the reoxygenation phase in more detail. Then, we assessed whether mean $r_s\text{SO}_2$ differed between intervention groups compared to the control group throughout the study using a Kruskal-Wallis test, with post hoc comparison to control. We repeated these steps for $r_s\text{CoVar}$. Finally, we assessed whether $r_s\text{SO}_2$ was associated with arterial partial pressure of oxygen (PaO_2) and arterial oxygen saturation (SaO_2) using linear regression analysis for pooled data throughout the experiment.

Results

We successfully collected complete data sets in 25 out of 42 piglets (Figure 2). We excluded 4 piglets that died, 2 piglets that reached a humane endpoint, and 14 that could not be completely evaluated because there were no NIRS monitors available. In 3 cases, there was a combination of these criteria. There were no differences in baseline characteristics among groups (Table 1).

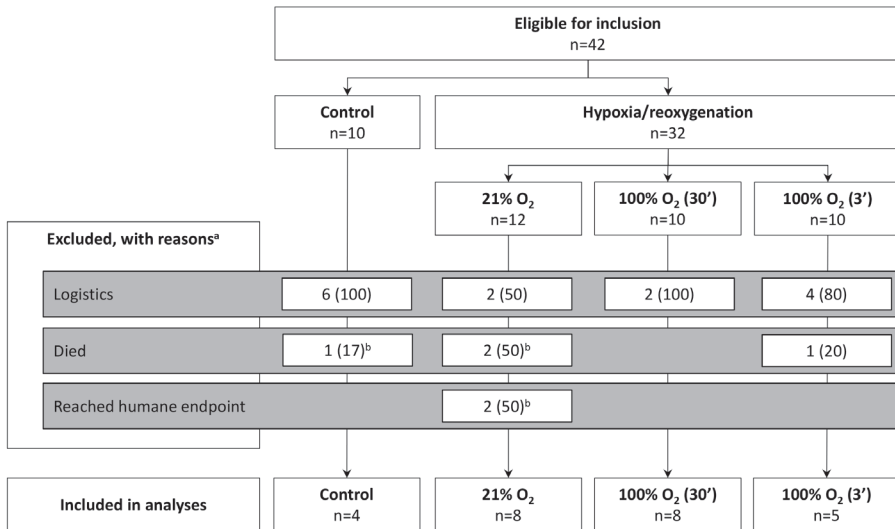


Figure 2. Study flowchart.

^a Presented as number (percentage). ^b In 3 cases there was a combination of these criteria.

The course of splanchnic oxygenation during hypoxia and reoxygenation

Mean r_sSO_2 was significantly lower during hypoxia compared to stabilization in the 21% O₂ group ($P=0.014$) (Figures 3a-d). Mean r_sSO_2 was significantly higher during reoxygenation compared to stabilization in the 100% O₂ (30') group ($P=0.002$). We found that in piglets in the 100% O₂ (30') and 100% O₂ (3') groups, r_sSO_2 had returned to stabilization values at 3 minutes after start of reoxygenation (Figures 4a-d). In piglets in the 100% O₂ (30') group, r_sSO_2 increased to values that were higher than during stabilization from 6 minutes after the start of reoxygenation onwards ($P<0.01$). In contrast, in piglets in the 21% O₂ group continued to have lower r_sSO_2 -values at 3 ($P=0.001$) and 6 minutes ($P=0.029$) after the start of reoxygenation compared to stabilization. Mean r_sSO_2 was higher during observation compared to stabilization in the 100% O₂ (30') and 100% O₂ (3') groups (Figures 3a-d). In the 100% O₂ (30') group, mean r_sSO_2 was significantly higher during the 8th ($P=0.020$) and 9th hour ($P=0.013$) of observation compared to stabilization. In the 100% O₂ (3') group, mean r_sSO_2 was significantly higher during the 4th ($P=0.019$), 8th ($P=0.014$), and 9th hour ($P=0.004$) of observation compared to stabilization.

Table 1. Physiological parameters of piglets exposed to induced hypoxia.

	Control (n=4)	21% O₂ (n=8)	100% O₂ (30') (n=8)	100% O₂ (3') (n=5)
Sex (male), n (%)	2 (50.0)	3 (37.5)	4 (50.0)	2 (40.0)
Weight (kg)	2.0 (0.1)	2.0 (0.1)	2.0 (0.1)	2.0 (0.1)
Duration of hypoxia (min)	-	35.5 (11.8)	29.3 (9.9)	31.4 (13.4)
Temperature (°C)				
Stabilization	38.9 (0.4)	39.0 (0.6)	39.4 (0.2)	39.0 (0.7)
Hypoxia	39.1 (0.7)	37.9 (0.7)	38.7 (0.4)	37.9 (1.1)
Reoxygenation	38.7 (0.7)	38.5 (0.8)	39.2 (0.4)	38.2 (0.8)
Study end	38.4 (1.0)	38.6 (0.5)	38.8 (0.5)	39.0 (0.3)
pH				
Stabilization	7.43 (0.13)	7.45 (0.05)	7.43 (0.05)	7.46 (0.02)
Hypoxia	7.30 (0.36)	6.96 (0.16)*	6.93 (0.14)*	6.98 (0.09)
Reoxygenation	7.54 (0.10)	7.19 (0.14)*	7.19 (0.11)*	7.25 (0.08)*
Study end	7.37 (0.07)	7.37 (0.10)	7.40 (0.08)	7.43 (0.07)
PaO ₂ (kPa)				
Stabilization	11.9 (4.7)	7.8 (0.8)*	9.1 (2.0)	8.6 (0.7)
Hypoxia	12.3 (5.2)	4.8 (0.6)*	4.9 (0.6)*	4.6 (0.5)*
Reoxygenation	8.7 (1.6)	10.3 (3.2)	43.2 (9.5)*	9.4 (1.8)
Study end	8.8 (2.5)	8.8 (1.6)	9.7 (3.9)	6.7 (3.8)
SaO ₂ (%)				
Stabilization	97.7 (2.5)	91.8 (4.1)*	93.6 (2.0)	94.8 (1.6)
Hypoxia	97.7 (2.5)	36.1 (9.7)*	32.3 (7.2)*	32.4 (6.1)*
Reoxygenation	95.7 (2.9)	85.9 (8.5)	100.0 (0.0)*	90.3 (8.4)
Study end	92.0 (5.6)	93.5 (5.3)	93.5 (3.6)	95.2 (4.8)
Hb (g/dL)				
Stabilization	8.3 (2.3)	7.7 (0.9)	7.5 (1.3)	6.6 (1.1)
Hypoxia	8.0 (2.2)	7.9 (1.0)	8.2 (1.3)	7.1 (1.2)
Reoxygenation	7.7 (2.2)	7.2 (0.8)	7.6 (1.4)	6.4 (1.4)
Study end	7.1 (1.5)	6.2 (1.1)	6.8 (1.6)	5.9 (1.0)

Data is presented as mean (SD) unless indicated otherwise.

Hb: hemoglobin, PaO₂: arterial partial pressure of oxygen, SaO₂: arterial oxygen saturation.

* $P < 0.05$ intervention groups vs. control.

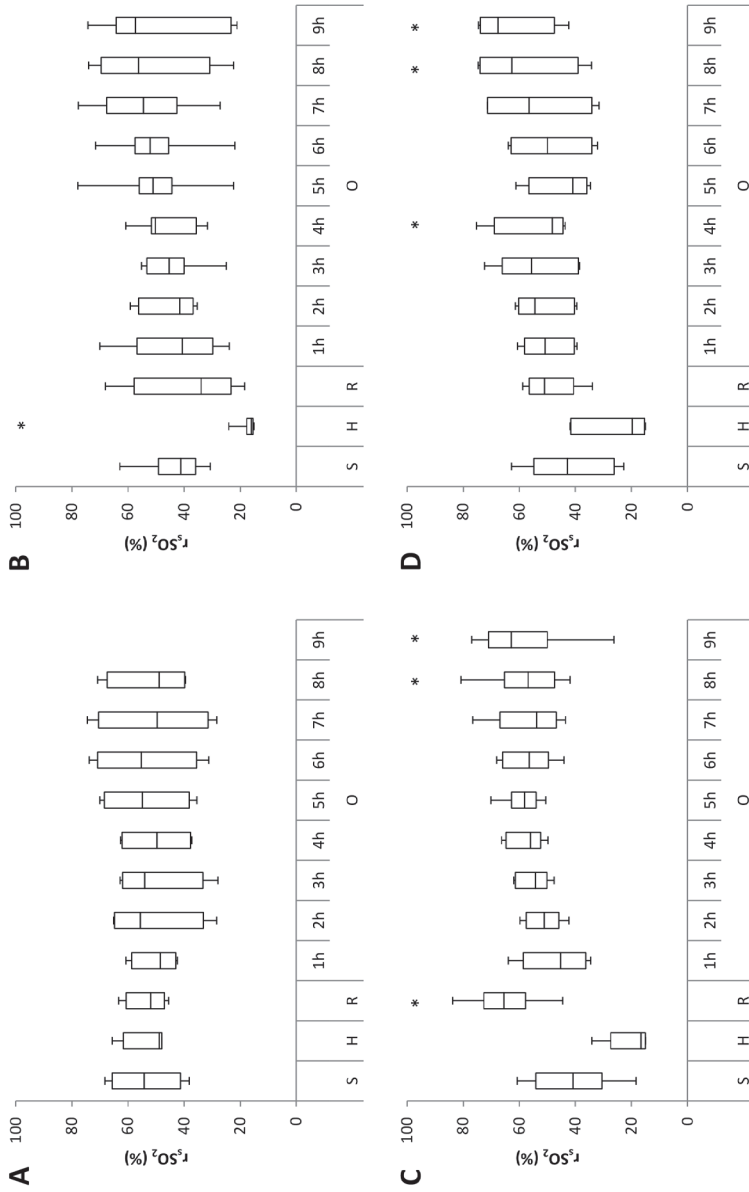


Figure 3. The course of regional splanchnic oxygen saturation throughout the study.
 A: Control, B: 21% O₂, C: 100% O₂ (30'), D: 100% O₂ (3').
 S: stabilization, H: hypoxia, R: reoxygenation, O: observation, h: hours.
 * *P*<0.05 compared to stabilization.

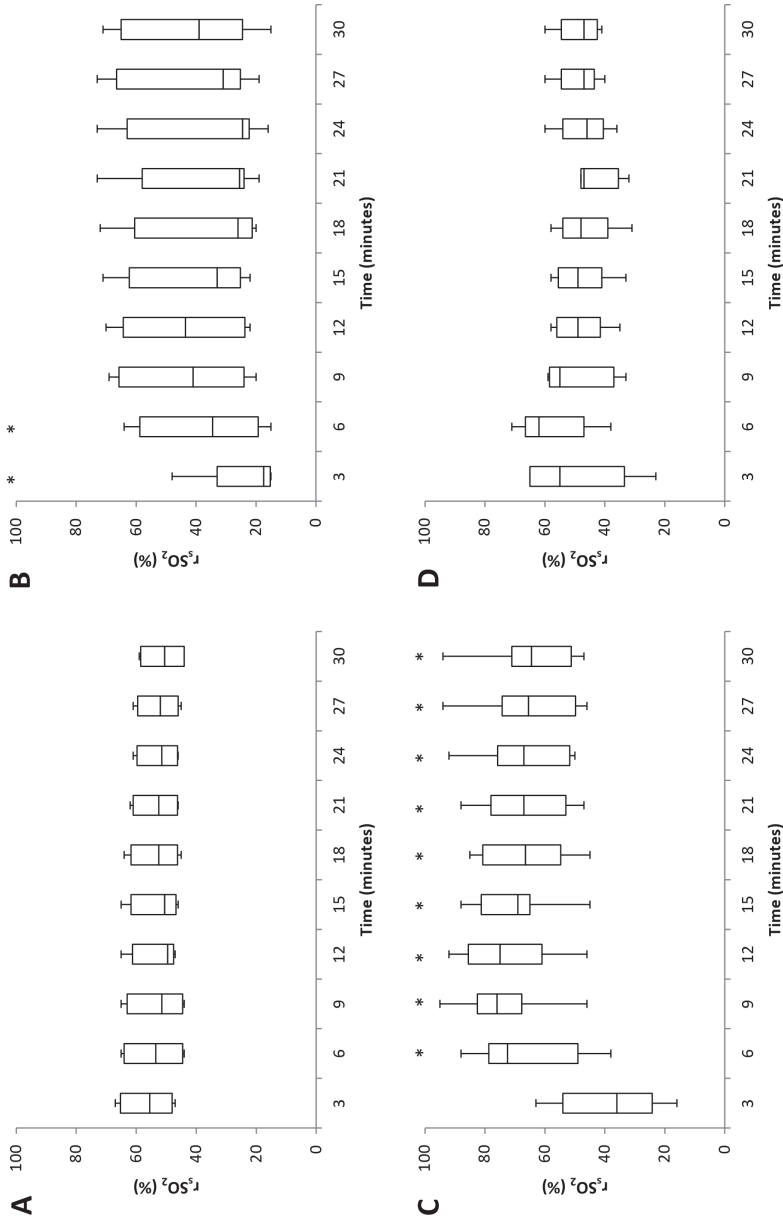


Figure 4. Regional splanchnic oxygen saturation during reoxygenation.
 A: Control, B: 21% O_2 , C: 100% O_2 (30'), D: 100% O_2 (3').
 * $P < 0.05$ compared to stabilization.

During hypoxia, mean $r_s\text{SO}_2$ was significantly lower in all intervention groups compared to the control group (21% O₂: $P=0.004$, 100% O₂ (30'): $P=0.003$, 100% O₂ (3''): $P=0.045$). During reoxygenation, mean $r_s\text{SO}_2$ at 3 minutes after the start of reoxygenation was lower compared to control in the 21% O₂ group ($P=0.004$). In the 100% O₂ (30') group, mean $r_s\text{SO}_2$ at 9 minutes after the start of reoxygenation was higher compared to control ($P=0.040$). We did not find any differences in mean $r_s\text{SO}_2$ between control and intervention groups during stabilization and observation.

Variability of splanchnic oxygenation

We found lower variability of $r_s\text{SO}_2$ during observation compared to stabilization in all intervention groups (Figures 5a-d). In the 21% O₂ group, $r_s\text{CoVar}$ was significantly lower during the 4th ($P=0.045$), 5th ($P=0.013$), and 8th hour ($P=0.035$) of observation compared to stabilization. In the 100% O₂ (30') group, $r_s\text{CoVar}$ was significantly lower during the 3rd to 9th hour of observation compared to stabilization ($P<0.05$). In the 100% O₂ (3'') group, $r_s\text{CoVar}$ was significantly lower during the 3rd ($P=0.039$) and 9th hour ($P=0.030$) of observation compared to stabilization.

During reoxygenation, $r_s\text{CoVar}$ was significantly higher in the 21% O₂ group compared to the control group ($P=0.009$). We did not find any differences in $r_s\text{CoVar}$ between control and intervention groups during stabilization, hypoxia, and observation.

Validation of splanchnic oxygenation monitoring

Finally, we assessed whether $r_s\text{SO}_2$ reflects changes in PaO₂ and SaO₂ (Figures 6a-b). We found that mean $r_s\text{SO}_2$ was correlated with PaO₂ ($r=0.420$, $P<0.001$) and SaO₂ ($r=0.648$, $P<0.001$) in pooled data throughout the experiment. These correlations were significant in both control and intervention groups (data not shown). However, when the phases of the experiments were analyzed separately, we only found significant correlations between $r_s\text{SO}_2$ and PaO₂ during hypoxia ($r=0.595$, $P<0.001$) and reoxygenation ($r=0.542$, $P<0.001$). Correlations between $r_s\text{SO}_2$ and SaO₂ were significant during hypoxia ($r=0.481$, $P<0.001$), reoxygenation ($r=0.553$, $P<0.001$), and observation ($r=0.191$, $P=0.016$).

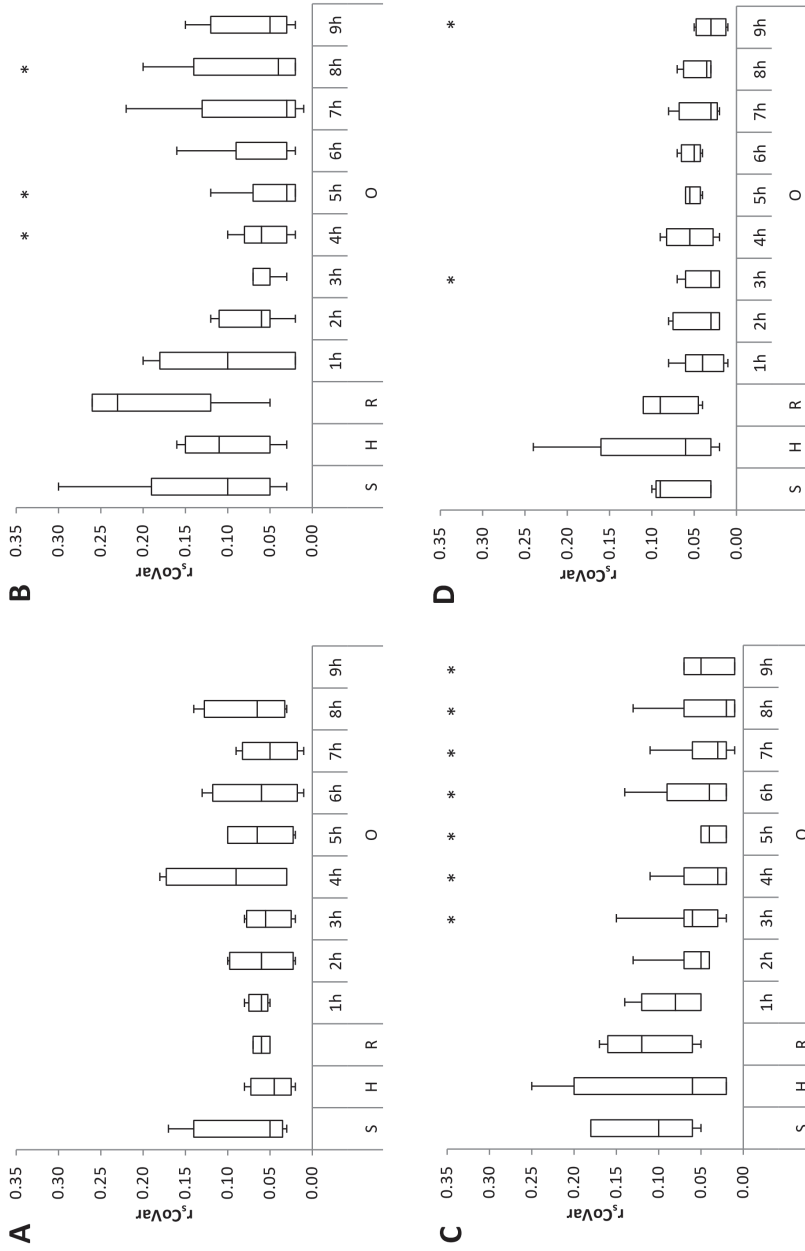


Figure 5. Variability of regional splanchnic oxygen saturation throughout the study.

A: Control, B: 21% O_2 , C: 100% O_2 (30'), D: 100% O_2 (3'').

S: stabilization, H: hypoxia, R: reoxygenation, O: observation, h: hours.

* $P < 0.05$ compared to stabilization.

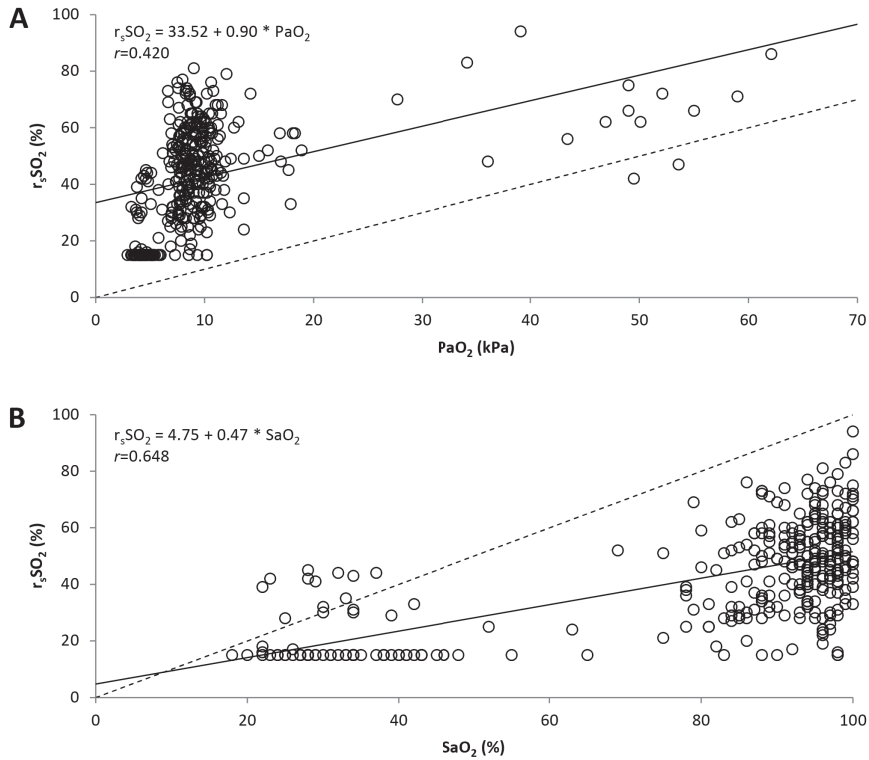


Figure 6. Correlation between regional splanchnic oxygen saturation and arterial oxygen content.

The solid line represents the regression line. The dotted line represents the line of identity.

Discussion

In this study, using a newborn piglet model of perinatal asphyxia, we assessed the effects of global hypoxia and different resuscitation strategies on splanchnic oxygenation. We demonstrated that reoxygenation following global hypoxia improves splanchnic oxygenation, although reoxygenation with 100% O₂ exposes the intestine to hyperoxia. In addition, we observed decreased variability of splanchnic oxygenation several hours after global hypoxia and subsequent reoxygenation, regardless of the applied resuscitation strategy. Finally, we found a strong association between splanchnic oxygenation and arterial oxygen content during hypoxia and reoxygenation.

To the best of our knowledge, we are the first to assess splanchnic oxygenation measured continuously and non-invasively with NIRS in a piglet model of asphyxia. Chen et al. have documented that r_sSO_2 decreases in a piglet model of graded whole-body

hypoxia and superior mesenteric artery ligation, but they did not assess $r_s\text{SO}_2$ during reoxygenation or reperfusion.¹⁴ In pigs that underwent hemorrhage and fluid resuscitation, changes in gastric tissue oxygen saturation have been studied, using a probe placed through gastrotomy.^{27,28} In these studies, an increase in gastric tissue oxygen saturation was observed during resuscitation. Similarly, Mallick et al. found an increase in intestinal tissue oxygen saturation, measured on the surface of the intestine, during reperfusion after intestinal ischemia in rats.²⁹ From these studies, we have learned that splanchnic oxygenation improves during resuscitation. Our results adds to the existing literature that $r_s\text{SO}_2$ improves upon reoxygenation after global hypoxia and that reoxygenation with 100% O_2 restores $r_s\text{SO}_2$ faster than reoxygenation with 21% O_2 , but also exposes the intestine to hyperoxia.

We found that variability of $r_s\text{SO}_2$ decreases several hours after global hypoxia and subsequent reoxygenation. Little is known about the physiological significance of variability of splanchnic oxygenation. We speculate that variability of $r_s\text{SO}_2$ indicates vascular adaptability to changes in oxygen supply and oxygen demand, similar to heart rate variability (HRV) that reflects changes of sympathetic and vagal activity.^{30,31} In preterm neonates, reduced HRV is associated with several pathologic conditions, including hypoxic-ischemic encephalopathy following perinatal asphyxia and NEC.³⁰⁻³² Similarly, lower baseline variability of $r_s\text{SO}_2$ and reduced $r_s\text{SO}_2$ -variability after RBC transfusion have been associated with NEC.^{20,33,34} Although the mechanisms leading to intestinal injury following anemia and RBC transfusion may differ from those following hypoxia and reoxygenation, we further speculate that reduced $r_s\text{SO}_2$ -variability may be indicative of microvascular injury, leading to impaired microvascular blood flow and increased microvascular permeability as seen in intestinal ischemia-reperfusion.³⁵ By definition, $r_s\text{CoVar}$ is dependent on mean $r_s\text{SO}_2$, thus, higher mean $r_s\text{SO}_2$ is accompanied by lower $r_s\text{CoVar}$. Indeed, in the intervention groups, mean $r_s\text{SO}_2$ gradually increased during the observation period. However, $r_s\text{CoVar}$ decreased disproportionately, an effect that was most pronounced in the 21% O_2 group. These results are in line with a recent meta-analysis on the use of high or low fraction of inspired oxygen in preterm infants in the delivery room.³⁶ Although no significant differences in rates of NEC were demonstrated, there was a trend towards a higher rate of NEC in the low oxygen group. As we observed reduced variability of $r_s\text{SO}_2$ in all intervention groups, but not the control group, intestinal microvascular injury may be a universal effect that occurs several hours after global hypoxia and reoxygenation, independent of the resuscitation strategy.

We verified that NIRS monitoring can accurately track splanchnic oxygenation during global hypoxia and reoxygenation by correlating $r_s\text{SO}_2$ with PaO_2 and SaO_2 . Our results confirm findings of previous studies that found an association between $r_s\text{SO}_2$ and arterial oxygen content.^{14,37} As we did not observe an association between $r_s\text{SO}_2$ and arterial oxygen content during stabilization and observation, we speculate that $r_s\text{SO}_2$ most accurately reflects changes in tissue oxygenation during substantial changes in arterial oxygen content. This is in line with previous findings by Brun et al. who demonstrated in newborn piglets that NIRS is less sensitive to cerebral tissue hypoxia during ischemic hypoxemia compared to hypoxic hypoxemia.³⁸ This contrast may be explained by the contribution of arterial vascular sources to tissue oxygen saturation measured by NIRS. While the difference between arterial and venous saturation increases during ischemia, this difference decreases during hypoxia and hyperoxia.^{39,40} This may explain why we found correlations between $r_s\text{SO}_2$ and arterial oxygen content during hypoxia and reoxygenation, but not during stabilization and observation. Our results thus suggest that splanchnic NIRS monitoring can detect exposure to hypoxia and hyperoxia most accurately during substantial changes in arterial oxygen content.

We found that piglets were exposed to intestinal hyperoxia during reoxygenation with 100% O₂ for 30 minutes, but not during reoxygenation with 100% O₂ for 3 minutes. Although the effects of hyperoxia on the intestine remain largely unknown, previous studies have shown that higher fractions of inspired oxygen lead to a dose-dependent increase in urinary levels of oxidative stress biomarkers.⁴¹ Nevertheless, some amount of oxygen supplementation seems to be required, as we found continued exposure to intestinal hypoxia during the first 6 minutes after start of reoxygenation with 21% O₂. Additional research is needed to provide more insight into the effects of different fractions of inspired oxygen and durations of hypoxia on oxidative stress, inflammatory markers, and intestinal injury.

Our study has several strengths. We used a well-established piglet model of perinatal asphyxia. Given the age of 6-36 hours of the piglets, our results best reflect postnatal hypoxia-reoxygenation. An important advantage of animal studies is the controlled, experimental set-up that allows for a systematic approach to the research question. In addition, piglets share many physiologic similarities to human neonates, in particular regarding the gastrointestinal tract.⁴² Our study had a randomized set-up that also included a control group, that allowed us to explore temporal changes within and between piglets. We also recognize some limitations. First, we had to exclude approximately 40% of the eligible piglets due to logistics, i.e., no NIRS monitor available. Only 7% of the eligible

piglets were excluded because they were unable to overcome the extreme conditions that resulted from the inflicted asphyxia, i.e., they died or reached a humane endpoint. As a result, the sample size of our study was small. In addition, we chose not to correct for multiple testing, due to the exploratory nature of this study. Both may have increased the type II error rate. Second, we observed lower baseline Hb in the 100% O₂ (3') group compared to control, although this did not reach statistical significance and did not seem to affect baseline r_sSO₂-values. Given the relative stability of Hb over time, this will not have affected changes in r_sSO₂, other than a potential underestimation of r_sSO₂ recovery or hyperoxia. Third, researchers who recorded the data, were not blinded to the applied resuscitation strategy. Finally, we did not perform histological analyses of intestinal samples. Future studies will need to address whether the observed changes in mean r_sSO₂ and variability of r_sSO₂ correlate with intestinal injury.

In conclusion, splanchnic NIRS monitoring was able to detect intestinal hypoxia and exposure to hyperoxia in a piglet model of asphyxia. Global hypoxia and subsequent reoxygenation was associated with reduced variability of r_sSO₂, that may be an indication of intestinal injury.

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