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BASIC SCIENCE ARTICLE

Splanchnic oxygen saturation during reoxygenation with 21% or 100% O$_2$ in newborn piglets

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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% O$_2$ 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 min with 21% or 100% O$_2$ and observed for 9 h. We continuously measured regional splanchnic oxygen saturation (r$_s$SO$_2$) using near-infrared spectroscopy (NIRS). We calculated mean r$_s$SO$_2$ and r$_s$CoVar (as SD/mean). We measured PaO$_2$ and SaO$_2$, sampled from the right carotid artery.

RESULTS: Reoxygenation after global hypoxia restored r$_s$SO$_2$. Reoxygenation with 100% O$_2$ increased r$_s$SO$_2$ to values significantly higher than baseline. In intervention groups, r$_s$CoVar decreased during observation compared to baseline. We found a correlation between r$_s$SO$_2$ and PaO$_2$ ($r = 0.420$, $P < 0.001$) and between r$_s$SO$_2$ and SaO$_2$ ($r = 0.648$, $P < 0.001$) in pooled data from the entire experiment.

CONCLUSION: Reoxygenation after global hypoxia improves splanchnic oxygenation, but is associated with reduced variability of r$_s$SO$_2$. Reoxygenation with 100% O$_2$ exposes the intestine to hyperoxia. Splanchnic NIRS is able to detect intestinal hypoxia and hyperoxia.

IMPACT:

- Splanchnic oxygenation improves during reoxygenation after global hypoxia, though reoxygenation with 100% O$_2$ exposes the intestine to hyperoxia.
- Decreased variability of splanchnic oxygenation several hours after hypoxia and reoxygenation seems to be independent of the resuscitation strategy, and may indicate intestinal injury.
- Splanchnic NIRS monitoring was able to detect intestinal hypoxia and exposure to hyperoxia, as evidenced by a strong correlation between splanchnic oxygenation and arterial oxygen content.

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, which 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 min 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$_s$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,

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and 75% venous.[8,9] Splanchnic NIRS monitoring was validated by correlating r_sSO_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.[14] 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. The response of r_sSO_2 to different resuscitation strategies following global hypoxia has not previously been studied, but it has been documented that r_sSO_2 increases in response to red blood cell (RBC) transfusions.[15–21] In addition, changes in variability of r_sSO_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_sSO_2 and variability of r_sSO_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 h. We excluded piglets with hemoglobin (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 h 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 and 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 min according to randomization, i.e. 21% O_2, 100% O_2, or 100% O_2 for 3 min followed by 21% O_2 for 27 min. 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 h after reoxygenation. We chose this duration for observation to partially include the secondary energy failure that starts approximately 6 h 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 Fig. 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).

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 regional splanchnic oxygen saturation (r_sSO_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_sSO_2 and the coefficient of variation for r_sSO_2 ($r_sCoVar = SD/mean$), as a measure of the variability of r_sSO_2. We used 5-min periods to pool and average $r_sCoVars$.[26]

Statistical analyses
For analyses, we divided the study in four phases, which are referred to as "stabilization", "hypoxia", "reoxygenation", and "observation" (Fig. 1). Given the long duration of observation, we analyzed this phase per hour. First, the normality of the data was assessed using the Kolmogorov−Smirnov test. Next, we assessed whether mean r_sSO_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_sSO_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_sCoVar_2. Finally, we assessed whether r_sSO_2 was associated with arterial oxygen pressure (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 (Fig. 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 three cases, there was a combination of these criteria. There were no differences in baseline characteristics among groups (Table 1).
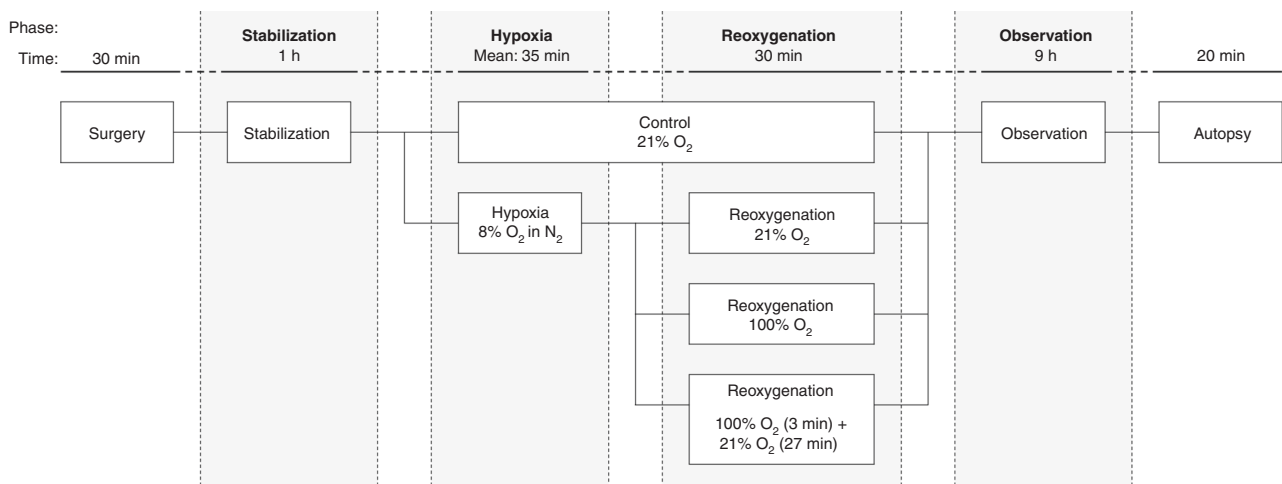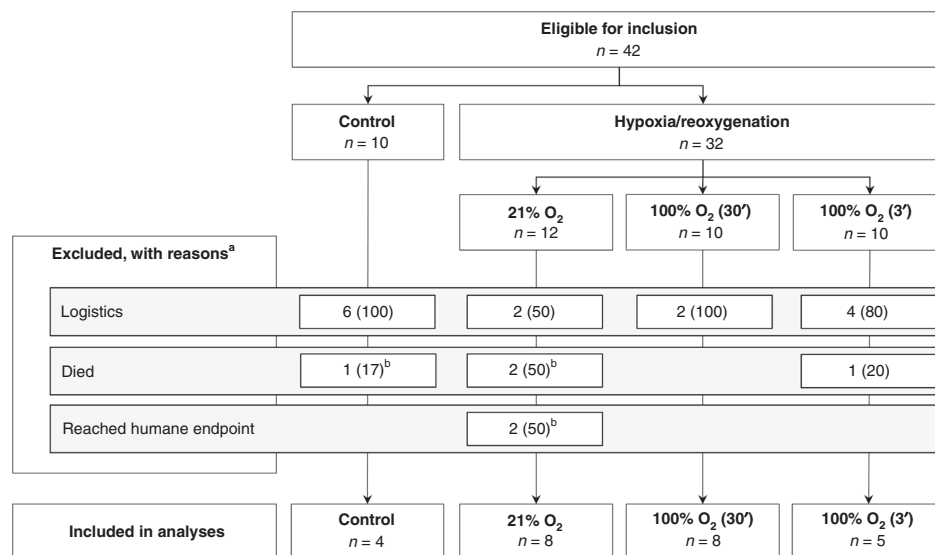

Fig. 1 Study protocol.

Fig. 2 Study flowchart. [a]Presented as number (percentage). [b]In three cases there was a combination of these criteria.

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

		Control (n = 4)	21% O$_2$ (n = 8)	100% O$_2$ (30′) (n = 8)	100% O$_2$ (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 are presented as mean (SD) unless indicated otherwise.
Hb hemoglobin.
*$P < 0.05$ intervention groups vs. control.

The course of splanchnic oxygenation during hypoxia and reoxygenation

Mean r$_s$SO$_2$ was significantly lower during hypoxia compared to stabilization in the 21% O$_2$ group ($P = 0.014$) (Fig. 3a–d). Mean r$_s$SO$_2$ was significantly higher during reoxygenation compared to stabilization in the 100% O$_2$ (30′) group ($P = 0.002$). We found that in piglets in the 100% O$_2$ (30′) and 100% O$_2$ (3′) groups, r$_s$SO$_2$ had returned to stabilization values at 3 min after the start of reoxygenation. In piglets in the 100% O$_2$ (30′) group, r$_s$SO$_2$ increased to values that were higher than during stabilization
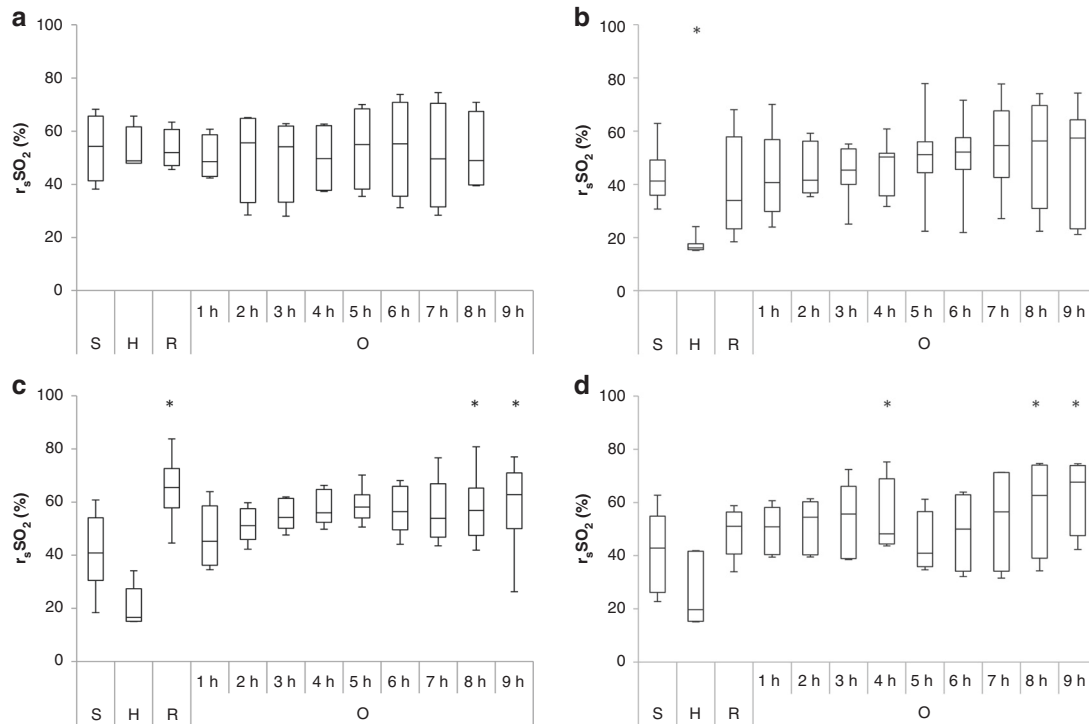
Fig. 3 **The course of regional splanchnic oxygen saturation throughout the study. a** Control, **b** 21% O_2, **c** 100% O_2 (30′), **d** 100% O_2 (3′). S stabilization, H hypoxia, R reoxygenation, O observation, h hours. *$P < 0.05$ compared to stabilization.

from 6 min after the start of reoxygenation onwards ($P < 0.01$). In contrast, in piglets in the 21% O_2 group continued to have lower r_sSO_2-values at 3 ($P = 0.001$) and 6 min ($P = 0.029$) after the start of reoxygenation compared to stabilization (Fig. 4a–d). Mean r_sSO_2 was higher during observation compared to stabilization in the 100% O_2 (30′) and 100% O_2 (3′) groups (Fig. 3a–d). In the 100% O_2 (30′) group, mean r_sSO_2 was significantly higher during the eighth ($P = 0.020$) and the ninth hour ($P = 0.013$) of observation compared to stabilization. In the 100% O_2 (3′) group, mean r_sSO_2 was significantly higher during the fourth ($P = 0.019$), eighth ($P = 0.014$), and ninth hour ($P = 0.004$) of observation compared to stabilization.

During hypoxia, mean r_sSO_2 was significantly lower in all intervention groups compared to the control group (21% O_2: $P = 0.004$, 100% O_2 (30′): $P = 0.003$, 100% O_2 (3′): $P = 0.045$). During reoxygenation, the mean r_sSO_2 at 3 min after the start of reoxygenation was lower compared to control in the 21% O_2 group ($P = 0.004$). In the 100% O_2 (30′) group, the mean r_sSO_2 at 9 min after the start of reoxygenation was higher compared to control ($P = 0.040$). We did not find any differences in mean r_sSO_2 between control and intervention groups during stabilization and observation.

Variability of splanchnic oxygenation
We found lower variability of r_sSO_2 during observation compared to stabilization in all intervention groups (Fig. 5a–d). In the 21% O_2 group, r_sCoVar was significantly lower during the fourth ($P = 0.045$), fifth ($P = 0.013$), and eighth hour ($P = 0.035$) of observation compared to stabilization. In the 100% O_2 (30′) group, r_sCoVar was significantly lower during the third to ninth hour of observation compared to stabilization ($P < 0.05$). In the 100% O_2 (3′) group, r_sCoVar was significantly lower during the third ($P = 0.039$) and ninth hour ($P = 0.030$) of observation compared to stabilization.

During reoxygenation, r_sCoVar was significantly higher in the 21% O_2 group compared to the control group ($P = 0.009$). We did

not find any differences in r_sCoVar between control and intervention groups during stabilization, hypoxia, and observation.

Validation of splanchnic oxygenation monitoring
Finally, we assessed whether r_sSO_2 reflects changes in PaO_2 and SaO_2 (Fig. 6a, b). We found that mean r_sSO_2 was correlated with PaO_2 ($r = 0.420$, $P < 0.001$) and SaO_2 ($r = 0.648$, $P < 0.001$) 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_sSO_2 and PaO_2 during hypoxia ($r = 0.595$, $P < 0.001$) and reoxygenation ($r = 0.542$, $P < 0.001$). Correlations between r_sSO_2 and SaO_2 were significant during hypoxia ($r = 0.481$, $P < 0.001$), reoxygenation ($r = 0.553$, $P < 0.001$), and observation ($r = 0.191$, $P = 0.016$).

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% O_2 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.[14] have documented that r_sSO_2 decreases in a piglet model of graded whole-body hypoxia and SMA ligation, but they did not assess r_sSO_2 during reoxygenation or reperfusion. In pigs that
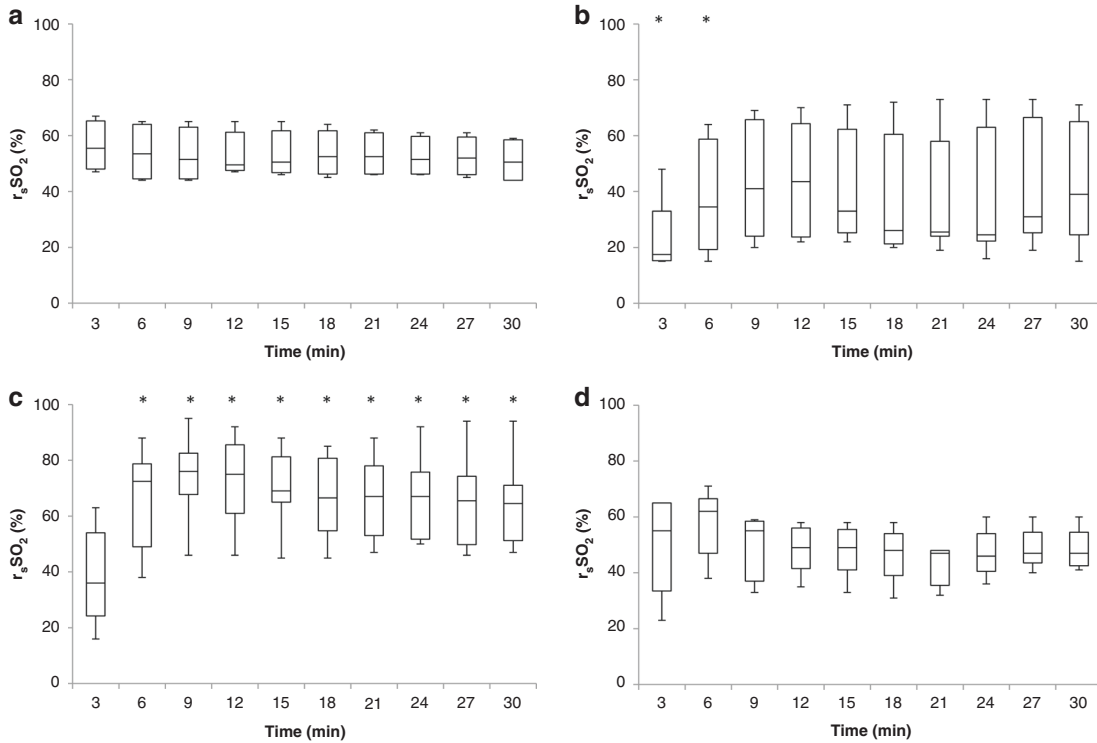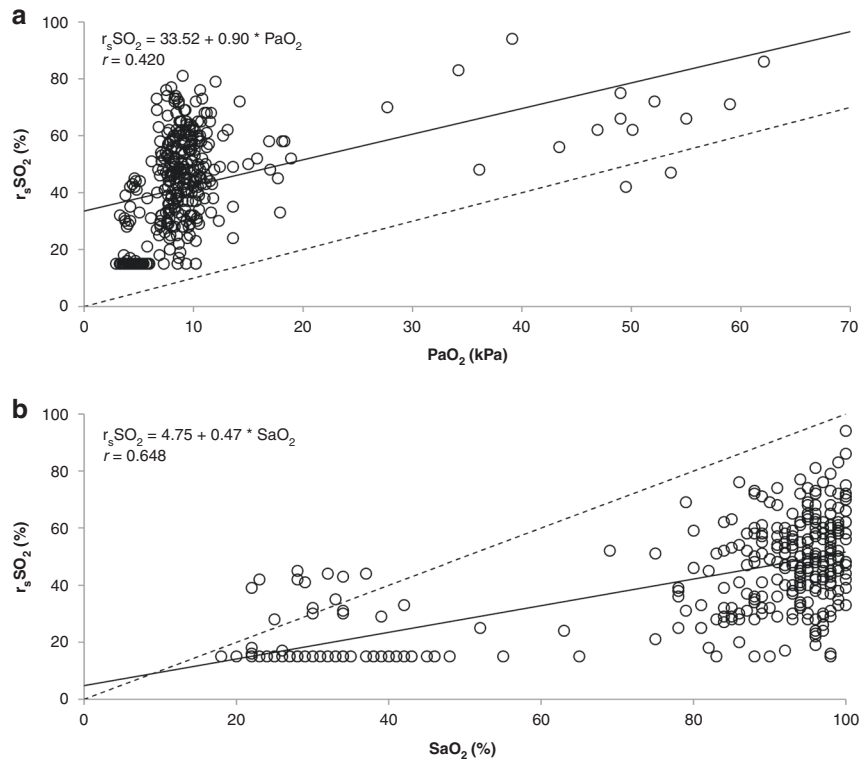
Fig. 4 Regional splanchnic oxygen saturation during reoxygenation. a Control, **b** 21% O_2, **c** 100% O_2 (30′), **d** 100% O_2 (3′). *$P < 0.05$ compared to stabilization.
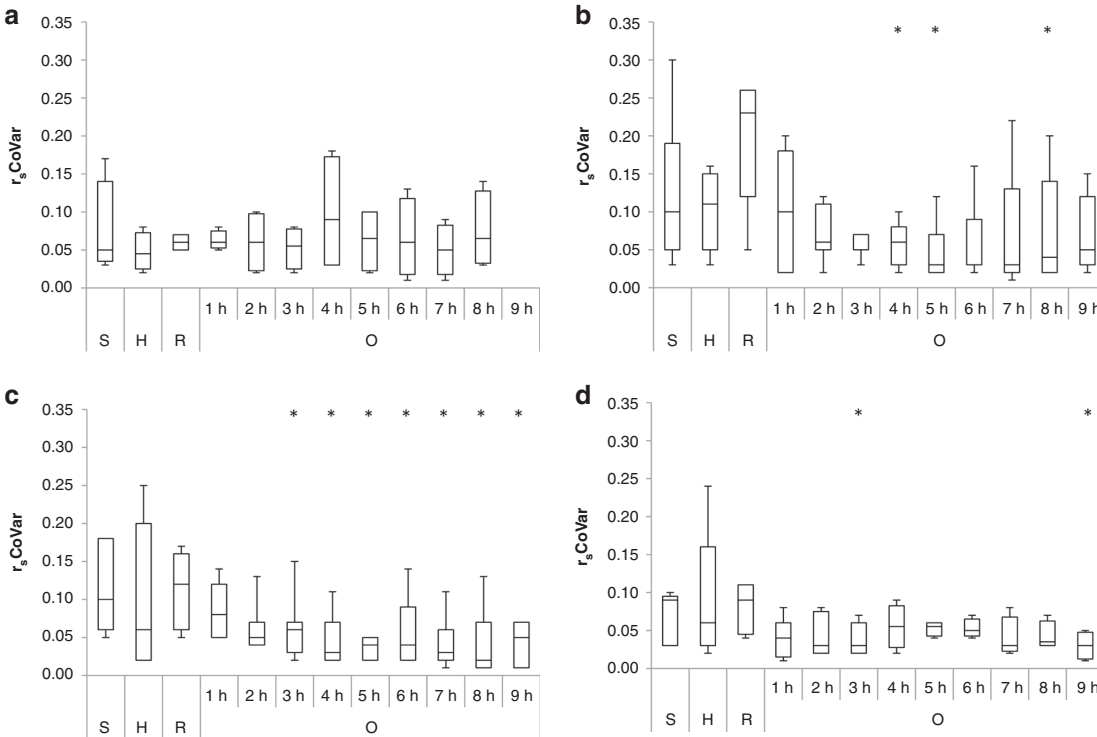
Fig. 5 Variability of regional splanchnic oxygen saturation throughout the study. a Control, **b** 21% O_2, **c** 100% O_2 (30′), **d** 100% O_2 (3′). S stabilization, H hypoxia, R reoxygenation, O observation, h hours. *$P < 0.05$ compared to stabilization.

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.[29] found an increase in intestinal tissue oxygen saturation, measured on the surface of the intestine, during reperfusion after intestinal ischemia in rats. From these studies, we have learned that splanchnic oxygenation improves

Fig. 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.

during resuscitation. Our results add to the existing literature that r_sSO_2 improves upon reoxygenation after global hypoxia and that reoxygenation with 100% O_2 restores r_sSO_2 faster than reoxygenation with 21% O_2, but also exposes the intestine to hyperoxia.

We found that variability of r_sSO_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_sSO_2 indicates vascular adaptability to changes in oxygen supply and oxygen demand, similar to heart rate variability (HRV) that reflects changes in 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_sSO_2 and reduced r_sSO_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_sSO_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_sCoVar is dependent on mean r_sSO_2; thus, higher mean r_sSO_2 is accompanied by lower r_sCoVar. Indeed, in the intervention groups, mean r_sSO_2 gradually increased during the observation period. However, r_sCoVar 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_sSO_2 in all intervention groups, but not the control group, an 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_sSO_2 with PaO_2 and SaO_2. Our results confirm the findings of previous studies that found an association between r_sSO_2 and arterial oxygen content.[14,37] As we did not observe an association between r_sSO_2 and arterial oxygen content during stabilization and observation, we speculate that r_sSO_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.[38] who demonstrated in newborn piglets that NIRS is less sensitive to cerebral tissue hypoxia during ischemic hypoxemia compared to hypoxic hypoxemia. 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_sSO_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% O_2 for 30 min, but not during reoxygenation with 100% O_2 for 3 min. 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 min after the start of reoxygenation with 21% O_2. 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 h of the piglets, our results best reflect postnatal hypoxia-reoxygenation. An important advantage of animal studies is the controlled, experimental setup 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 setup that also included a control group, which 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% O_2 (3′) group compared to control, although this did not reach statistical significance and did not seem to affect baseline r_sSO_2-values. Given the relative stability of Hb over time, this will not have affected changes in r_sSO_2, other than a potential underestimation of r_sSO_2 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_sSO_2 and variability of r_sSO_2 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 were associated with reduced variability of r_sSO_2, which may be an indication of intestinal injury.

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B.M.D., E.M.W.K., R.S., and O.D.S. were involved in the design and execution of the study. All authors were involved in the final consensus process of the protocol and revised the manuscript critically for important intellectual content. B.M.D. drafted the manuscript and all other authors read, edited, and approved the final manuscript for publication.

COMPETING INTERESTS
The authors declare no competing interests.

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